(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/18032 A2

(51) International Patent Classification[7]: C07K 5/00

(21) International Application Number: PCT/US00/24658

(22) International Filing Date:
8 September 2000 (08.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/394,962	10 September 1999 (10.09.1999)	US
09/539,935	31 March 2000 (31.03.2000)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	09/394,962 (CON)
Filed on	10 September 1999 (10.09.1999)
US	09/539,935 (CON)
Filed on	31 March 2000 (31.03.2000)

(71) Applicants *(for all designated States except US)*: **BASF AKTIENGESELLSCHAFT** [DE/DE]; Rheinland-Pfalz, D-67056 Ludwigshafen (DE). **ARIZONA BOARD OF REGENTS** [US/US]; Arizona State University, Tempe, AZ 85287-3511 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PETIT, George, R.** [US/US]; 6232 Bret Hills Drive, Paradise Valley, AZ 85253 (US). **SRIRANGAM, Jayaram, K.** [IN/US]; 9395 Pipilo Street, San Diego, CA 92129 (US). **WILLIAMS, Michael, D.** [US/US]; 1544 South Vine, Gilbert, AZ 85233 (US). **DURKIN, Kieran, P., M.** [IE/US]; 110 Kerwin Way, Folsom, CA 95630 (US). **BARLOZZARI, Teresa** [IT/US]; 24 South Woodside Avenue, Wellesley, MA 02181 (US). **KLING, Andreas** [DE/DE]; Riegeler Weg 14, D-68239 Mannheim (DE). **JANSSEN, Bernd** [DE/US]; 114 Applebriar Lane, Marlborough, MA 01752 (US). **HAUPT, Andreas** [DE/DE]; Schaelzigweg 52, D-68723 Schwetzingen (DE).

(74) Agents: **ELMORE, Carolyn, S.** et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02421 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

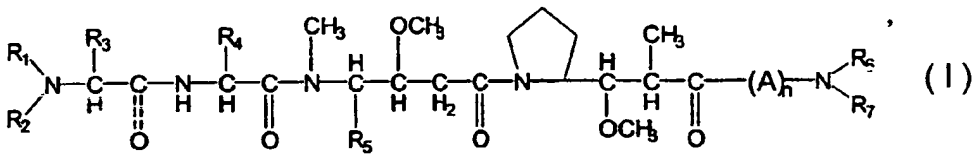
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DOLASTATIN PEPTIDES



(I)

(57) Abstract: The present invention provides compounds of formula (I) where R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group; A is a methionyl, phenylalanyl or phenylglycyl residue; n is 0 or 1; R_6 is a hydrogen atom; and R_7 is a carbocyclic group, an aromatic group, a C_1-C_4-alkyl group, a pyridylalkyl group or a heterocyclic group. In another embodiment, R_6 is benzyl or -C(O)R_8, where R_8 is a C_1-C_6-alkyl group, and R_7 is a heteroaromatic group, such as a 2-thiazolyl group.

WO 01/18032 A2

DOLASTATIN PEPTIDES

RELATED APPLICATION

This application is a Continuation of U.S. Application No.: 09/539,935, filed March 31, 2000 which is a Continuation of U.S. Application No.: 09/394,962, filed September 10, 1999, the entire teachings of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

A series of short peptides with significant activity as cell growth inhibitors have been isolated from the Indian Ocean sea hare *Dolabella auricularia* (Pettit *et al., J. Am. Chem. Soc.* **109** : 6883-6885 (1987); Beckwith *et al., J. Natl. Cancer Inst.* **85**, 483-88 (1993); United States Patent No. 4,816,444; European Patent Application Publication No. 398558). These peptides are referred to as Dolastatins 1-15. Of these, Dolastatins 10 and 15 are the most potent cell growth inhibitors. Dolastatin 15, for example, inhibits the growth of the National Cancer Institute's P388 lymphocytic leukemia (PS system) cell line, a strong predictor of efficacy against various types of human malignancies. Dolastatin 10 and Dolastatin 15 effectively inhibit tubulin polymerization and growth of four different human lymphoma cell lines (Bai *et al., Biochem. Pharmacol.* **39** : 1941-1949 (1990); Beckwith *et al., supra* (1993)).

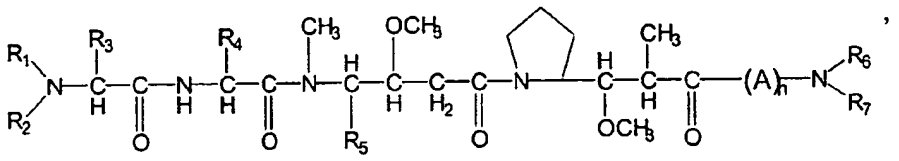
The minute amounts of the Dolastatin peptides present in *Dolabella auricularia* (about 1 mg each per 100 kg sea hare) and the consequent difficulties in purifying amounts sufficient for evaluation and use, have motivated efforts toward the synthesis of the more promising of these compounds, including Dolastatin 10 (Pettit *et al., J. Am. Chem Soc.* **111**: 5463-5465 (1989); Roux *et al. Tetrahedron* **50** : 5345-5360 (1994); Shiori *et al. Tetrahedron* **49** : 1913-1924 (1993)). Synthetic Dolastatin 10, however, suffers from disadvantages which include poor solubility in aqueous systems and the need for expensive starting materials for its synthesis.

These disadvantages, in turn, have led to the synthesis and evaluation of structurally modified Dolastatin 10 derivatives.

A need persists for synthetic compounds with the biological activity of Dolastatin 10 which have useful aqueous solubility and can be produced efficiently and economically.

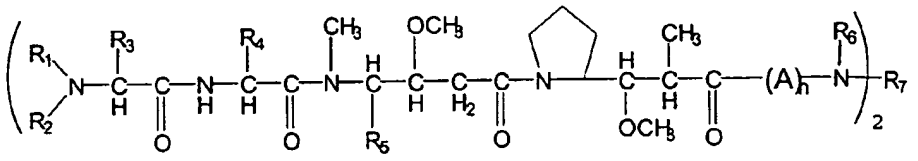
SUMMARY OF THE INVENTION

The present invention provides compounds of the formula



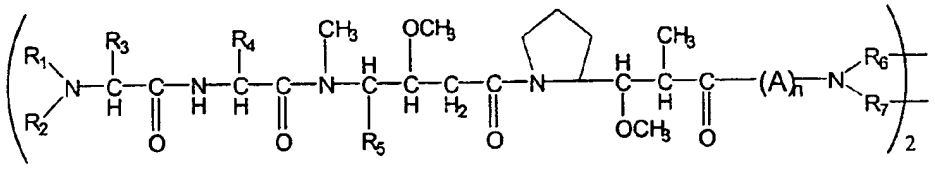
where R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group; A is a methionyl, phenylalanyl or phenylglycyl residue; n is 0 or 1; R_6 is a hydrogen atom; and R_7 is a carbocyclic group, an aromatic group, a straight chain or branched C_1-C_4-alkyl group, a pyridylalkyl group or a heterocyclic group. In another embodiment, R_6 is benzyl or $-C(O)OR_8$, where R_8 is a C_1-C_6-alkyl group, and R_7 is a heteroaromatic group, such as a 2-thiazolyl group.

In another embodiment, the invention relates to compounds of the formula

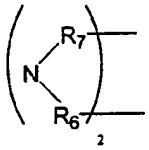


where R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group; A is a methionyl, phenylalanyl or phenylglycyl residue; n is 0 or 1; R_6 is a hydrogen atom; and R_7 is an aromatic group.

In yet another embodiment, the invention provides compounds of the formula



where R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group; A is a methionyl, phenylalanyl or phenylglycyl residue; n is 0 or 1; and



is a five- or six-membered ring.

In yet another embodiment, the present invention provides a method for treating cancer in a patient. The method comprises the step of administering to the patient a therapeutically effective amount of a compound of the invention. The invention also relates to the use of a compound of the invention for the manufacture of a medicament for treating cancer in a patient.

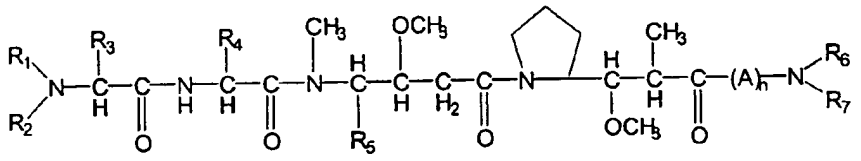
DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to peptides having antineoplastic activity. It also includes pharmaceutical compositions comprising these compounds and methods for treating cancer in a mammal, including a human, by administration of these compositions to the mammal.

Dolastatin 10, a peptide isolated from the sea hare *Dolabella auricularia*, is a potent inhibitor of cell growth. This compound, however, is present in trace quantities in the sea hare, and is thus difficult to isolate. Dolastatin 10 is also expensive to synthesize and suffers from poor aqueous solubility. As shown herein, however, Dolastatin 10 can serve as a starting point for the development of compounds which overcome these disadvantages while retaining antineoplastic activity or exhibiting greater antineoplastic activity than the natural product.

Applicants have discovered that certain structural modifications of Dolastatin 10 provide compounds with a surprisingly improved therapeutic potential for the treatment of neoplastic diseases as compared to Dolastatin 10. Furthermore, the compounds of the present invention can be conveniently synthesized, as described below in detail.

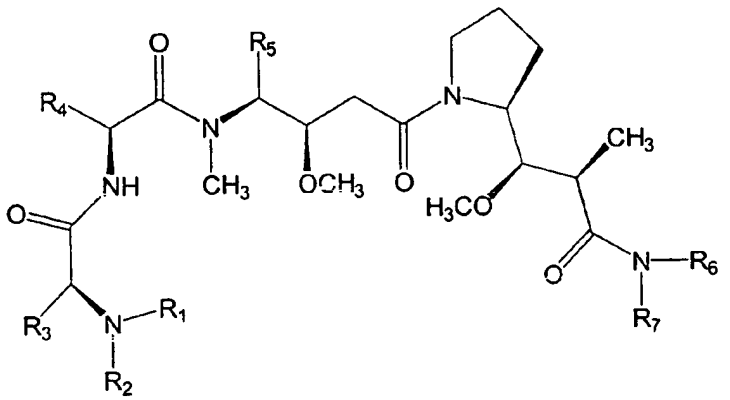
The present invention provides antitumor peptides of Formula I,



(I),

where R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group. A is a methionyl, phenylalanyl or phenylglycyl residue and n is 0 or 1. In one embodiment, R_6 is a hydrogen atom and R_7 is a carbocylic group, an aromatic group, a C_1-C_4-alkyl group, a pyridylalkyl group or a heterocyclic group. In another embodiment, R_6 is benzyl or -C(O)OR_8, where R_8 is a C_1-C_6-alkyl group, and R_7 is a heteroaromatic group, such as a 2-thiazolyl group.

The peptides of Formula I are generally composed of L-amino acids but they can also contain one or more D-amino acids. Preferred compounds of the invention are of Formula I and have the stereochemistry indicated below for a peptide of Formula I wherein n=0.

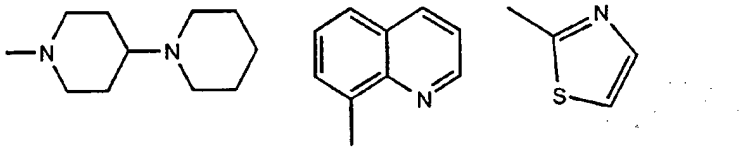


In the following discussion, compounds of Formula I have the stereochemistry shown above unless otherwise indicated.

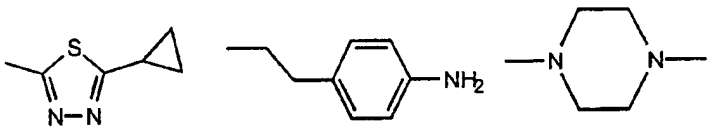
The present compounds can also exist as salts with pharmaceutically-acceptable acids, including hydrochloric acid, citric acid, tartaric acid, lactic acid, phosphoric acid, methanesulfonic acid, acetic acid, formic acid, maleic acid, fumaric acid, malic acid, succinic acid, malonic acid, sulfuric acid, L-glutamic acid, L-aspartic acid, pyruvic acid, mucic acid, benzoic acid, glucuronic acid, oxalic acid, ascorbic acid and acetylglycine.

In preferred embodiments, R_1 and R_2 are each methyl, R_3 is an isopropyl or sec-butyl group, R_4 is an isopropyl, sec-butyl or isobutyl group, and R_5 is sec-butyl.

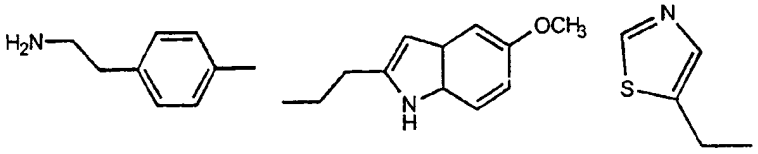
In one embodiment, R_6 is a hydrogen atom and R_7 is selected from among methyl, t-butyl, isopropyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-(3-pyridyl)ethyl, 4-pyridyl and groups a-r, shown below. These and other groups depicted herein are identified by the appropriate letter in Tables 1-11.



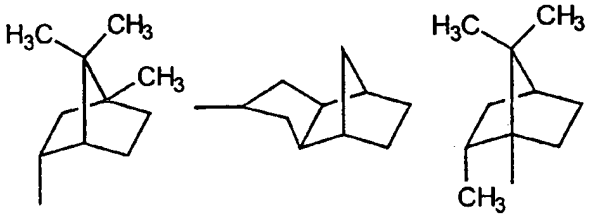
(a) (b) (c)



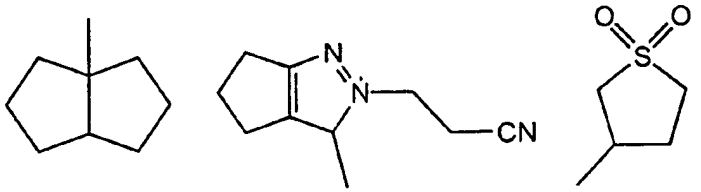
(d) (e) (f)



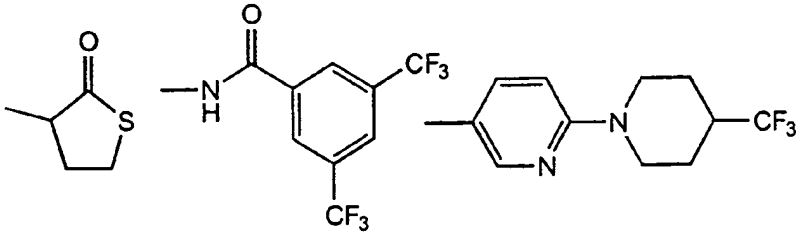
(g) (h) (i)



(j) (k) (l)



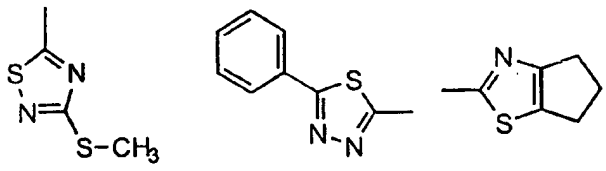
(m) (n) (o)



(p) (q) (r)

In another embodiment, R_6 is $-C(O)OCH_3$ or benzyl and R_7 is 2-thiazolyl.

One subset of compounds of the present invention include pentapeptides of formula I wherein R_1 and R_2 are each methyl, R_3 is isopropyl, R_4 is isopropyl, R_5 is sec-butyl, n is 1, A is a methionyl residue, R_6 is a hydrogen atom and R_7 is selected from among the groups j, k, m and n, shown above, and groups s, t and u, below..



(s) (t) (u)

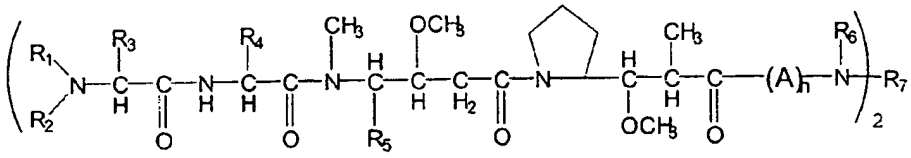
Another subset of the compounds of the present invention include tetrapeptides of Formula I in which R_1 and R_2 are each methyl, R_3 and R_4 are each isopropyl, R_5 is sec-butyl, n is 0, R_6 is a hydrogen atom and R_7 is selected from among t-butyl, isopropyl, methyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-(3-pyridyl)ethyl, and 4-pyridyl, or R_7 is selected from among groups k, l, m, o, p q and r.

Another subset of compounds of the present invention includes tetrapeptides of Formula I wherein R_1 and R_2 are each methyl, R_3 is isopropyl, R_4 and R_5 are each sec-butyl, n is 0, R_6 is a hydrogen atom and R_7 is selected from among groups s and t.

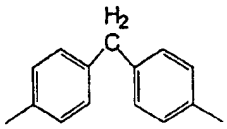
Another subset of the compounds of the present invention includes tetrapeptides of Formula I in which R_1 and R_2 are each methyl, R_3 is isopropyl, R_4 is isopropyl or sec-butyl, R_5 is sec-butyl, n is 0, R_6 is a benzyl group or $-C(O)OCH_3$ and R_7 is a 2-thiazolyl group.

Another subset of compounds of the invention include pentapeptides of Formula I wherein R_1 and R_2 are each methyl, R_3 is isopropyl, R_4 is isopropyl, R_5 is sec-butyl, n is 1, A is a phenylalanyl residue, R_6 is a hydrogen atom and R_7 is selected from among groups s and t.

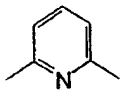
The invention also provides compounds in which two peptides are linked. In one embodiment, R_7 is a bridging group, for example an aromatic group or an arylalkyl group, which links the C-terminal amide nitrogen atoms of two peptides as shown below.



In this formula, R_1-R_6, A and n are as defined in Formula I above. Suitable examples of R_7 in such compounds groups u and v, shown below.

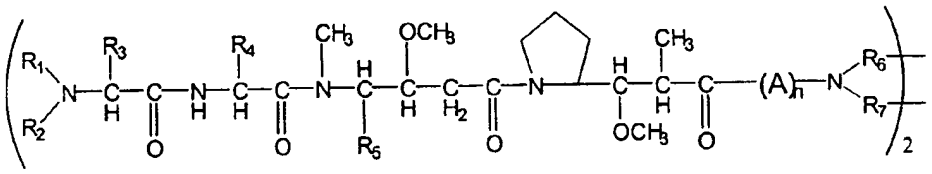


(v)



(w)

In another embodiment, the invention provides compounds of the formula



wherein R_1-R_5, A and n are as defined in Formula I and R_6, R_7 and the C-terminal amide nitrogen atoms of two peptides form a five or six-membered ring. For example, R_6 and R_7 can each be a methylene group. In this case, the two C-terminal amide nitrogen atoms are linked by two ethylene groups.

The compounds of the invention can be synthesized using conventional methods of synthetic peptide chemistry, as described in the Examples and depicted in Schemes I-VIII. For example, synthesis of the pentapeptides of the invention can proceed via an amino acid amide of the formula A-N(R_6)R_7, where A is methionine, phenylalanine or phenylglycine, which can be prepared by coupling the N-Boc (Boc = t-butoxycarbonyl) protected amino acid with the appropriate primary or secondary amine. The resulting amino acid amide can then be deprotected with trifluoroacetic acid and coupled with N-Boc-dolaproine to produce the corresponding dipeptide amide. The dipeptide amide can then be deprotected with trifluoroacetic acid and the resulting trifluoroacetate salt of the free amine can be coupled with an appropriate tripeptide trifuoroacetate salt.

The tetrapeptides of the invention can be prepared via a similar route. N-Boc-dolaproine can be reacted with an appropriate primary or secondary amine to form a N-Boc-dolaproine amide. The N-Boc-dolaproine amide can then be

deprotected with trifluoroacetic acid, and the resulting trifuoroacetate salt of the free amine can be coupled with the appropriate tripeptide trifluoroacetate salt.

The coupling reactions can be performed by treating the peptides with a coupling agent, such as EDC with dimethylaminopyridine, ethyl chloroformate with N-methylmorpholine, or diethyl phosphorocyanidate with triethylamine. The coupling reactions are generally performed in an inert solvent, such as dichloromethane or tetrahydrofuran. The reaction temperature is typically from about -10°C to room temperature, preferably about 0°C. The segments to be coupled are generally reacted in about equimolar amounts. About 1 to 1.2 equivalents of the coupling agent can be used, in combination with about 2 to about 4 equivalents of the amine. The deprotection of the N-Boc group can be performed with an acid, such as trifluoroacetic acid, in an inert solvent, such as dichloromethane.

In another embodiment, the present invention comprises a method for partially or totally inhibiting formation of, or otherwise treating (e.g., reversing or inhibiting the further development of) solid tumors (e.g., tumors of the lung, breast, colon, prostate, bladder, rectum, or endometrial tumors) or hematological malignancies (e.g., leukemias, lymphomas) in a mammal, for example, a human, by administering to the mammal a therapeutically effective amount of a compound or a combination of compounds of Formula I. The compound(s) may be administered alone or in a pharmaceutical composition comprising the compound(s) and an acceptable carrier or diluent. The compound or compounds of Formula I can also be administered in combination with one or more additional therapeutic agents, such as anti-cancer chemotherapeutic agents. The compound or compounds of Formula I can be administered simultaneously with the additional agent(s), or the administration of the compound(s) of Formula I and the additional agent(s) can be offset by a suitable period of time, such as hours. Administration can be by any of the means which are conventional for pharmaceutical, preferably oncological, agents, including oral and parenteral means, such as subcutaneously, intravenously, intramuscularly and intraperitoneally, nasally or rectally. The compounds may be administered alone or in the form of pharmaceutical compositions containing a compound or compounds of Formula I together with a pharmaceutically accepted

carrier appropriate for the desired route of administration. Such pharmaceutical compositions may be combination products, i.e., they may also contain other therapeutically active ingredients.

The dosage to be administered to the mammal, such as a human, will contain a therapeutically effective amount of a compound described herein. As used herein, a "therapeutically effective amount" is an amount sufficient to inhibit (partially or totally) formation of a tumor or a hematological malignancy or to reverse development of a solid tumor or other malignancy or prevent or reduce its further progression. For a particular condition or method of treatment, the dosage is determined empirically, using known methods, and will depend upon factors such as the biological activity of the particular compound employed; the means of administration; the age, health and body weight of the recipient; the nature and extent of the symptoms; the frequency of treatment; the administration of other therapies; and the effect desired. A typical daily dose will be from about 0.05 to about 50 milligrams per kilogram of body weight by oral administration and from about 0.01 to about 20 milligrams per kilogram of body weight by parenteral administration.

The compounds of the present invention can be administered in conventional solid or liquid pharmaceutical administration forms, for example, uncoated or (film-)coated tablets, capsules, powders, granules, suppositories or solutions. These are produced in a conventional manner. The active substances can for this purpose be processed with conventional pharmaceutical aids such as tablet binders, fillers, preservatives, tablet disintegrants, flow regulators, plasticizers, wetting agents, dispersants, emulsifiers, solvents, sustained release compositions, antioxidants and/or propellant gases (cf. H. Sücker *et al.*: Pharmazeutische Technologie, Thieme-Verlag, Stuttgart, 1978). The administration forms obtained in this way typically contain from about 1 to about 90% by weight of the active substance.

The present invention will now be illustrated by the following examples, which are not to be considered limiting in any way.

EXAMPLES

Example 1 - Synthesis of N-Boc amino acid amides, 3a-e

General Procedure A

To a solution of N-Boc amino acid 1 (4.01 mmol) in anhydrous dichloromethane (20 mL) was added at -10°C, under argon, triethylamine (4.01 mmol, 1.0 equiv.), followed by ethylchloroformate (4.01 mmol, 1.0 equiv.). After stirring at -10°C for 40 min, the amine (2, 4.01 mmol, 1.0 equiv.) in anhydrous dichloromethane (20 ml) was added and the stirring continued at -10°C for an additional 1 hr. The solvent was removed *in vacuo* and replaced by ethyl acetate and the triethylamine hydrochloride salt was removed by filtration. The filtrate was concentrated under reduced pressure and the residue subjected to flash chromatography using suitable eluents to obtain the required amino acid amides 3.

Synthesis of N-tert-Butoxycarbonylmethionine 1-amino-bicyclo[3.3.0]octane amide

Reaction of N-Boc-L-methionine (1.0 g, 4.01 mmol, 1.0 equiv.) with 1-aminobicyclo[3.3.0]octane (2d) following General Procedure A gave, following isolation, a residue which was subjected to silica gel column chromatography (hexane:ethyl acetate, 1:1) to yield a colorless solid which was recrystallized from dichloromethane/n-hexane to afford the required product as colorless needles (3d, 900 mg, 63%); $[\alpha]^{25}_{D}$= -11.5° (c 1.42, $CHCl_3$); mP 152-153°C; IR(film): 3304, 3067, 1684, 1651 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.26 (2H, sextet, *J* 6.1, 12.18 Hz), 1.44 (9H, s, Boc), 1.60 (4H, pentet, *J* 6.7 Hz), 1.76 (2H, pentet, *J* 6.7, 5.55 Hz), 1.89-2.07(6H, m), 2.11 (3H, s, SMe), 2.32(1H, heptet), 2.54 (2H, m), 4.14(1H, q), 5.19(1H, brd, NH), 6.30(1H, s, NH); MS(m/z): 356(M^+, 5%), 300, 282, 226, 149, 119, 104 and 57 (100%).

Table 1. Physical constants and spectroscopic data for the Boc-amino acid amides 3a-e

no.	R	R_7	yield %	mp °C	$[\alpha]_D^{25}$°, $CHCl_3$	ir, ν_{max}, cm^{-1}	^1H nmr, δ	ms M^+
3a	$(CH_2)_2SMe$	k	83	oil	-5.3 (c 1.78)	3297 1690 1680 1659	1.10(2H,q), 1.27-1.58(7H, m), 1.47(9H, s), 1.89(4H, m), 2.04(1H,m), 2.11(3H, s), 2.27(2H, m), 2.55(2H, m), 4.21(2H, m), 5.21(1H, brd), 6.25(1H, brd)	382
3b	$(CH_2)_2SMe$	j	93	89-93	-49 (c 1.44)	3329 1692 1659	0.83(3H, s), 1.1-1.31(2H, m), 1.44(9H, s), 1.56(2H, m), 1.65-1.75(2H, m), 1.85(1H, dd), 1.87-2.15(2H, m), 2.11(3H, s), 2.56(2H, m), 3.87(1H, dt), 4.18(1H, q), 5.16(1H, brd), 6.29(1H, brd)	384
3c	$(CH_2)_2SMe$	n	44	177-178	-47 (c 0.29)	3333 3281 2284 1676	1.46(9H, s), 2.04(1H, m), 2.14(3H, s), 2.21(1H, m), 2.37(2H, m), 2.57-2.71(6H, m), 2.88(2H, t), 4.18(2H, t), 4.38(1H, q), 5.20(1H, d), 8.23(1H, brs)	407
3e	Ph	Ph	85	134-135	-105 (c 0.53)	3329 1686 1663	1.43(9H, s), 5.33(1H, brs), 5.79(1H, brs), 7.08(1H, t), 7.24-7.46(9H, m), 7.74(1H, s)	326

Example 2 - Deprotection of N-Boc-amino acid amides 3a-e

General Procedure B

A solution of the Boc-amino acid amide 3a-e (1.0 mmol) in dry dichloromethane (10 ml)/trifluoroacetic acid (2.0 ml) was stirred at 0°C for 3 hr under argon. The solvent was removed *in vacuo* and the reside dried under high vacuum for 2 hr. The oily trifluoroacetate salts 4a-e obtained were used without further purification in the coupling reaction.

Example 3 - Synthesis of dipeptide amides 6a-e

General Procedure C

The amino acid amide trifluoroacetate salt 4 (1.0 mmol) was dissolved in anhydrous dichloromethane (15 ml) and the solution cooled to 0°C. Triethylamine (10.7 mmol, 11 equiv.) was added followed by diethyl phosphorocyanidate (DEPC, 1.2mmol, 1.2 equiv.) and the mixture was stirred for 2-8 hr at 0°C. The solvent was removed *in vacuo* and the residue was purified by silica gel flash chromatography to yield the respective dipeptide amides 6a-e.

Synthesis of N-tert-butoxycarbonyl-dolaproine-methionine 1-aminobicyclo[3.3.0] octane amide, 6d

Reaction of the trifluoroacetate salt 4d with Boc-dolaproine (5) using General Procedure C gave a residue which was purified by silica gel flash chromatography (hexane-ethyl acetate-methanol, 2:2:0.1) to afford a colorless solid (6d, 41%); $[\alpha]_D^{25}$= -49° (c 0.82, $CHC1_3$); IR(film): 3285, 2949, 2868, 1694, 1640, 1549, 1397, 1173, 1105 cm^{-1}; ^1H NMR (300 MHz, $CDC1_3$) δ: 1.24(5H, m), 1.48(9H, s, Bu^t), 1.59(4H, m), 1.74(4H, m), 1.93(8H, m), 2.11(3H, s), 2.31(2H, m), 2.49(1H, m), 2.60(1H, m), 3.19-3.27(1H, m), 3.40 and 3.55(1H, m), 3.43(3H, s), 3.76(1H, m), 3.85(1H, m), 4.45(1H, m), 6.44(1H, brs), 6.58 and 6.81(1H, brs); MS(m/z): 525(M^+ 4%), 493, 451, 419, 408, 393, 356, 341, 312, 210, 171, 154, 139 and 115 (100%).

Table 2. Physical constants and spectroscopic data for the Boc-Dap-amino acid amides 6a-e

no.	R	R_7	yield %	mp °C	$[\alpha]_D^{25}$°, $CHCl_3$	ir, ν_{max}, cm^{-1}	^1H nmr, δ	ms, M^+
6a	$(CH_2)_2SMe$	k	88	-	-44 (c 0.26)	3277 1698 1676 1626	1.10(1H, m), 1.25(3H, dd), 1.30-1.53(7H, m), 1.48(9H, s), 1.65-2.10(9H, m), 2.12(3H, s), 2.26(1H, m), 2.32-3.0(5H, m), 3.27(1H, m), 3.43(3H, s), 3.46(1H, m), 3.81(2H, m), 4.06-4.32(2H, m), 4.50(1H, q), 6.6/6.9(1H, brs)	551
6b	$(CH_2)_2SMe$	j	36	-	-93.5 (c 0.17)	1695 1637 1545	0.79(3H, s), 0.82(3H, s), 1.09(3H, s), 1.24(5H, m), 1.43, 1.46, 1.49(9H, s), 1.34-1.60(2H, m), 1.67-2.02(8H, m), 2.10(3H, s), 2.40-2.65(3H, m), 3.20-3.27(2H, m), 3.44(3H, s), 3.55(1H, m), 3.83(2H, m), 3.92(1H, m), 4.49(1H, m), 6.50(1H, m), 6.7/7.1(1H, d)	553
6c	$(CH_2)_2SMe$	n	53	-	-62 (c 1.18)	2251 1684 1645 1537	1.27(3H, m), 1.32(9H, s), 1.47(1H, brm), 1.66-2.0(6H, m), 2.12(3H, s), 2.32(4H, m), 2.52(4H, m), 2.64(4H, t), 2.85(2H, m), 3.26/ 3.50(2H, m), 3.44(3H, s), 3.8(2H, m), 4.16(2H, t), 4.67(1H, m), 7.19(1H, d), 8.66/8.95(1H, s)	576
6e	Ph	Ph	21	204	-119 (c 0.18)	3306 3277 1698 1642	1.23(3H, d), 1.42(9H, s), 1.71(3H, m), 1.85(2H, m), 1.93(1H, m), 2.49(1H, t), 3.19(1H, m), 3.39(3H, s), 3.45, 3.83(1H, brs), 3.81(H, m), 5.61(1H, m), 7.08(1H, t), 7.25-7.36(6H, m), 7.44(3H, m), 7.80(1H, brs)	463 (M^+-CH_3OH)

Example 4 - Deprotection of Boc-dipeptide amides 6a-e

General Procedure D

A solution of the Boc-dipeptide amide (6a-e, 0.1 mmol) in dry dichloromethane (2 ml)/trifluoroacetic acid (1 ml) was stirred at 0°C for 2 hr under argon. The solvent was removed *in vacuo* and the residue dissolved in toluene and reconcentrated. The oily trifluoroacetate salts (7a-e) thus obtained were dried under high vacuum and used without further purification in the next coupling reaction. The general procedures of Examples 1-4 are depicted in Scheme I.

Example 5 - Synthesis of Boc-Dolaproine amides 9a-g

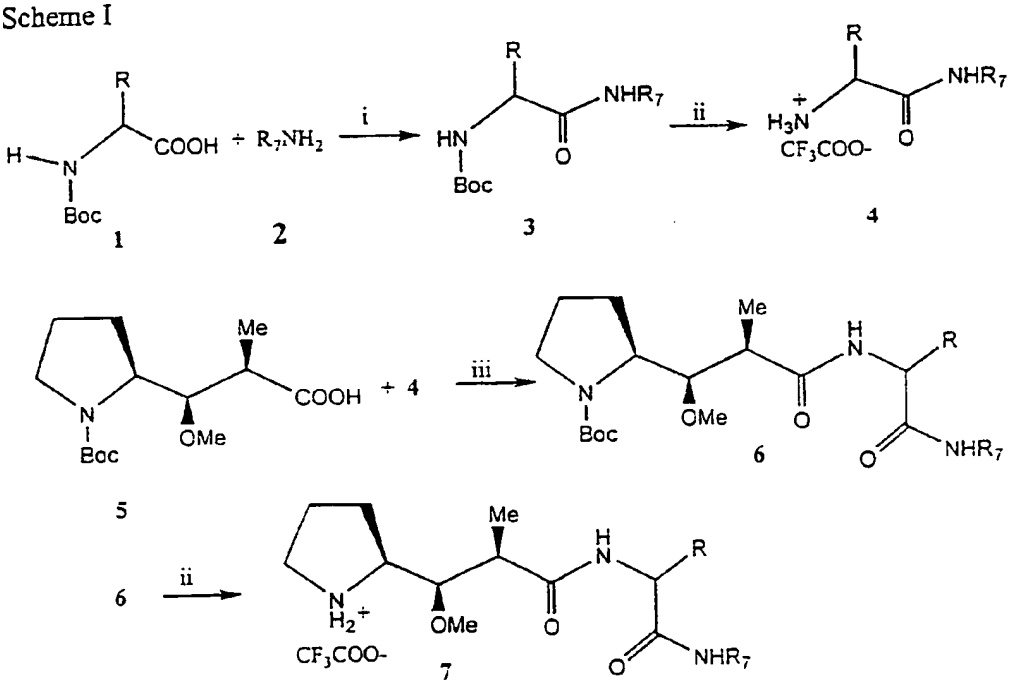
General Procedure E

To a solution of N-Boc-dolaproine 5 (1.74 mmol, 1.0 equiv.) in anhydrous THF (20 ml) cooled to 0°C, was added 1-hydroxybenzotriazole (1.74 mmol, 1.0 equiv.), triethylamine (0.24 ml, 1.74 mmol, 1.0 equiv.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1.74 mmol, 1.0 equiv.) and the reaction mixture was stirred at 0°C for 1hr. The amine (8, 1.74 mmol, 1.0equiv.) was added and the reaction was stirred at 0°C for 1 hr and at room temperature for 12 hr. Ethyl acetate (50 ml) was added and the solution was sequentially washed with aqueous sodium bicarbonate (7%, 30 ml), water (30 ml) and brine (30 ml). After drying over sodium sulfate the solvent was removed *in vacuo* and the residue subjected to silica gel column chromatography to afford the required amide 9.

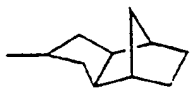
General Procedure F

To a stirred solution of Boc-dolaproine 5 (1.74 mmol) in anhydrous dichloromethane (10 ml) cooled to -10°C, was added triethylamine (1.74 mmol, 1.0 equiv.) followed by isobutyl chloroformate (1.74 mmol, 1.0 equiv.) and the reaction was continued at -10°C for 30 min. The amine (8a-g, 1.74 mmol, 1.0 equiv.) was added and the reaction mixture stirred at -10°C for 2 hr. The solvent was removed *in vacuo*, and the residue was dissolved in ethyl acetate. Triethylamine hydrochloride was collected by filtration and the filtrate was concentrated *in vacuo*. The residue was subjected to silica gel column chromatography to afford the required amides 9a-g.

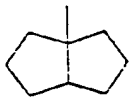
Scheme I



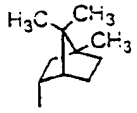
a) R = -(CH$_2$)$_2$SMe, R$_7$ =



d) R = -(CH$_2$)$_2$SMe, R$_7$ =

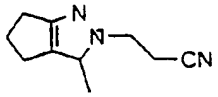


b) R = -(CH$_2$)$_2$SMe, R$_7$ =



e) R = phenyl, R$_7$ = phenyl

c) R = -(CH$_2$)$_2$SMe, R$_7$ =



i) ethyl chloroformate, triethylamine, dichloromethane

ii) trifluoroacetic acid, dichloromethane

iii) diethylphosphorocyanidate (DEPC), triethylamine, dichloromethane

Synthesis of N-tert-butoxycarbonyl-dolaproine 1-amino-bicyclo[3.3.0]octane amide 9b

Reaction of Boc-dolaproine (5) in anhydrous THF (20 ml) with 1-aminobicyclo[3.3.0]octane (8b) following General Procedure E gave a residue which was subjected to silica gel chromatography (eluent hexane-ethyl acetate; 4:1) to afford a colorless oil (9b, 64%); $[\alpha]_D^{25}$= -40° (c 0.45, chloroform); IR(film): 3339, 2936, 1693, 1682, 1667, 1643 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.21(3H, d, *J* 5Hz), 1.23-1.29(2H, m), 1.48(9H, s, Bu^t), 1.55-2.01(14H, m), 2.10-2.45(2H, m), 3.26(1H, m), 3.33-3.65(1H, dm), 3.44(3H, s, OMe), 3.68-3.80(2H, dm), 5.68/6.39(1H, s, H); MS (m/z): 394(M^+, 0.1%), 362, 321, 262, 225, 210, 170, 154, 114(100%), 70(100%) and 57.

Example 6 - Deprotection of the Boc-Dolaproine amides 9a-g

General Procedure G

A solution of the Boc-dolaproine amide (9a-g, 0.1 mmol) in dry dichloromethane (2 ml)-trifluoroacetic acid (1.0 ml) was stirred at 0°C for 2 hr under argon. The solvent was removed *in vacuo* and the residue taken up in toluene and reconcentrated. The oily trifluoroacetate salts (10a-g) obtained were dried under high vacuum and used without further purification in the next coupling reaction. The general procedures of Examples 5 and 6 are depicted in Scheme II.

Example 7 - Synthesis of the pentapeptide amides 12a-e

General Procedure H

To a solution of the above trifluoroacetate salt of the dipeptide amide (7a-e, 0.1 mmol) or the trifluoroacetate salt of the dolaproine amide (10a-g, 0.1 mmol) and the tripeptide trifluoroacetate salt (Tfa* Dov-Val-Dil-COOH, 11, 0.1 mM) in dry dichloromethane (2 ml) cooled to ice-bath temperature under argon was added triethylamine (3-4 eq.) followed by DEPC (1.2 eq.) and the solution was stirred at the same temperature for 2 hr. The solvent was removed *in vacuo* and the residue chromatographed on a silica gel column to provide the respective pentapeptide amides (12a-e) or the tetrapeptide amides (13a-g). This procedure is depicted in Scheme III.

Table 3. Physical constants and spectroscopic data for the Boc-Dap-amides 9a-g

no.	R_7	Yield %	mp °C	$[\alpha]_D^{25°}$, $CHCl_3$	ir, ν_{max}, cm^{-1}	^1H nmr, δ	ms, M^+
9a	o	92	-	-30 (c 0.72)	3321 1815 1737 1693 1645	1.26(3H, d), 1.47(9H, s), 1.64(1H, m), 1.79(2H, m), 1.94(2H, m), 2.26-2.42(2H, m), 2.51(1H, m), 3.09(2H, m), 3.30(2H, m), 3.40(2H, m), 3.45(3H, s), 3.79(2H, d), 4.69(1H, m)	372
9b	m	27	-	-18 (c 1.18)	1691 1689 1662	1.26(3H, d), 1.47(9H, s), 1.53-2.05(6H, m), 2.50(1H, m), 2.88(1H, m), 3.21-3.41(4H, m), 3.45(3H, s), 3.83(1H, brd), 3.90(1H, m), 4.58(1H, m)	354
9c	p	63	174-180	-49 (c 0.5)	3227 1684 1642	1.29(3H, d), 1.45(9H, s), 1.72-2.04(4H, m), 2.62(1H, m), 2.89/2.97(1H, s), 3.25(1H, m), 3.48(4H, s), 3.94(2H, m), 7.98(1H, s), 8.32(2H, s)	541
9e	j	50	-	-69 (c 1.02)	3350 1694 1672	0.84(3H, s), 0.85(3H, s), 0.93(3H, m), 1.10-1.34(6H, m), 1.48(9H, s), 1.50-2.02(8H, m), 2.20-2.5(1H, m), 3.26(1H, m), 3.30-3.60(1H, m), 3.43(3H, s), 3.8(1H, brm), 3.86(2H, brm), 5.67/6.15(1H, brs)	422
9f	k	43	-	-38 (c 0.44)	3308 1694 1670 1643	1.00-1.39(5H, m), 1.41-1,57(4H, m), 1.48(9H, s), 1.65-2.02(6H, m), 2.10(2H, m), 2.10-2.50(4H, m), 3.27(1H, m), 3.45(3H, s), 3.33-3.63(1H, brd), 3.70-3.90(2H, brm), 5.67/6.15(1H, brs)	420
9g	r	57	-	-30 (c 0.66)	1668 1665	1.33(3H, m), 1.48(9H, s), 1.65(4H, m), 1.79(1H, m), 1.94(4H, d), 2.24(1H, m), 2.65(1H, m), 2.80(2H, t), 3.27(1H, m), 3.39-3.60(1H, m), 3.50(3H, s), 3.92(2H, m), 4.34(2H, d), 6.67(1H, d), 7.6-8.37(3H, m)	514

Synthesis of Dov-Val-Dil-Dap-Met 1-(bicyclo[3.3.0]octane) amide (12d)

Reaction of the trifluoroacetate 7d with tripeptide trifluoroacetate 11 following General Procedure G gave, following chromatography (silica gel column using 3:1 acetone-hexane as eluent), the required pentapeptide amide as a colorless glassy solid (12d, 94%); R_f = 0.55(dichloromethane-methanol 8:1); $[\alpha]_D^{25}$= -36.5° (c 0.17, chloroform); mP 95-102 °C; IR(thin film): 3574, 3509, 3493, 3476, 3293, 3059, 2959, 2936, 2878, 2832, 1643, 1622, 1547, 1539, 1504, 1445, 1416, 1385, 1371, 1337, 1283, 1271, 1223, 1198, 1167, 1036 cm^{-1}; ^1H NMR(300MHz, $CDCl_3$, partial assignment): 6.98(d), 6.9(d), 6.56(s), 4.76(m), 4.40(q), 4.26(m), 4.09(m), 3.92(dd), 3.38(s), 3.30(s), 3.00(s) and 2.09(s); MS{m/z(%)}: 836(M^+), 793, 763, 684, 611, 481, 412, 227, 186(100) and 170; Anal. Found: C 61.97, H 9.34, N 9.71; $C_{44}H_{80}N_6O_7S.H_2O$ requires: C 61.79, H 9.66, N 9.83%.

Example 8 - Synthesis of the tetrapeptide amides 13a-g

Dov-Val-Dil-Dap 1-bicyclo[3.3.0]octane amide (13b)

Coupling the trifluoroacetate 10b with the tripeptide trifluoroacetate 11 according to General Procedure H, followed by chromatography (silica gel column) of the residue in 2:1 acetone-hexane, gave the required tetrapeptide amide (13b, 89%) as a colorless glassy solid; R_f = 0.61 (3:2 acetone-hexane); $[\alpha]_D^{25}$= -44° (c 0.17, $CHCl_3$); mP 97-102°C; IR(thin film): 3308, 2959, 2936, 2872, 2830, 1622, 1534, 1489, 1451, 1418, 1385, 1371, 1339, 1267, 1217, 1200, 1132, 1099 and 1038 cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$, partial assignment): 6.92(m), 6.31(s), 4.86(m), 4.76(q), 4.04-4.15(m), 3.38(s), 3.32(s), 3.30(s), 3.08(s), 2.99(s), 2.28-2.40(m), 1.56(pentet); MS(m/z): 705(M^+), 662, 525, 481, 449, 379, 293, 227, 199, 186 and 155(100%). This procedure is depicted in Scheme IV.

Example 9 - Synthesis of N-t-Boc amino acid amides 16a-g

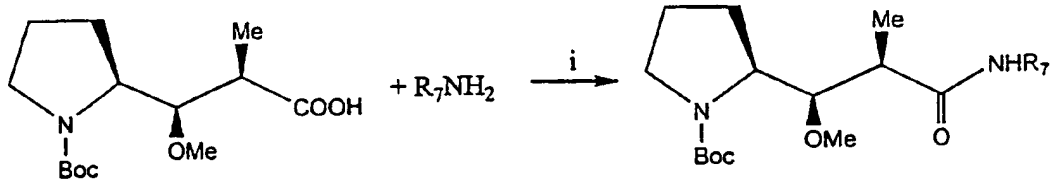
Synthesis of t-Boc-phenylalanine amide 16d

A solution of N-t-Boc-Phenylalanine (1g, 3.77 mM) in anhydrous tetrahydrofuran (25ml) was cooled to -15°C and neutralized with N-methylmorpholine (450 µl). Isobutyl chloroformate (550 µl) was added followed by 3-amino-(5-thiomethyl)thia-1,4-diazole (2i, 550 mg, 3.77 mM). The reaction mixture was allowed to warm to room temperature. After stirring for 1h, the

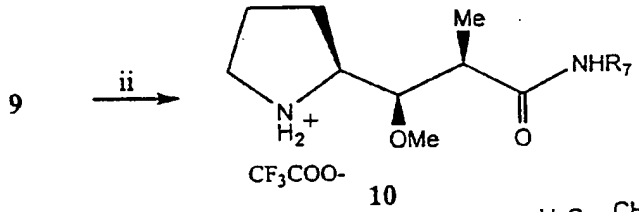
Table 4. Physical constants and spectroscopic data for the pentapeptide amides 12a-e

no.	R	R_7	yield %	mp °C	R_f	$[\alpha]_D^{25}$ °CHCl_3	ir, νmax, cm^{-1}	^1H nmr, δ	ms M^+	Molecular Formula
12a	$(CH_2)_2SMe$	k	77	114-120	0.46 (3:2 acetone-hexane)	-54 (c 0.19)	3293 1641 1626	6.99(d), 4.76(t), 4.45(m), 4.1(m), 3.31(s), 3.3(s), 3.25(s), 3.0(s), 2.99(s), 2.27(s), 2.1(s), 2.09(s)	862	$C_{46}H_{82}N_6O_7S.3H_2O$
12b	$(CH_2)_2SMe$	j	84	98-103	0.54 (3:2 acetone-hexane)	-83 (c 0.06)	3293 1647 1624	7.21(d), 6.53(d), 4.75(t), 4.43(q), 4.19(m), 4.09(m), 3.38(s), 3.29(s), 3.0(s), 2.09(s)	864	$C_{46}H_{84}N_6O_7S.3H_2O$
12c	$(CH_2)_2SMe$	n	96	-	0.48 (8:1 dichloro-methane methanol)	-34.5 (c 0.29)	3395 2280 1643 1624	7.49(d), 4.7(m), 4.23(m), 3.92(m), 3.41(s), 3.28(s), 2.96(s), 2.46(bs), 2.13(s), 1.36(t)	887	$C_{45}H_{78}N_9O_9S$
12e	Ph	Ph	94	-	-	-87.5 (c 0.12)	3290 1643 1622	7.54(m), 7.46(m), 7.31(m), 7.07(m), 6.92(d), 5.2-6.4(d), 4.75(m), 4.12(m), 3.87, 3.98(m), 3.73(m), 3.56(dd), 3.28(m), 3.32(s), 3.37, 3.39(s), 3.20(d), 2.99, 3.11(s)	806	$C_{45}H_{70}N_6O_7.H_2O$

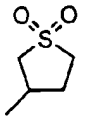
Scheme II

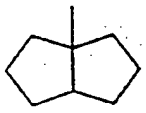


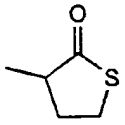
5 8 9

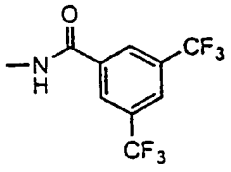


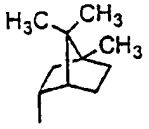
10

a) R_7 = 

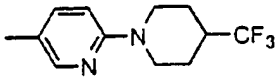
b) R_7 = 

c) R_7 = 

d) R_7 = 

e) R_7 = 

f) R_7 = 

g) R_7 = 

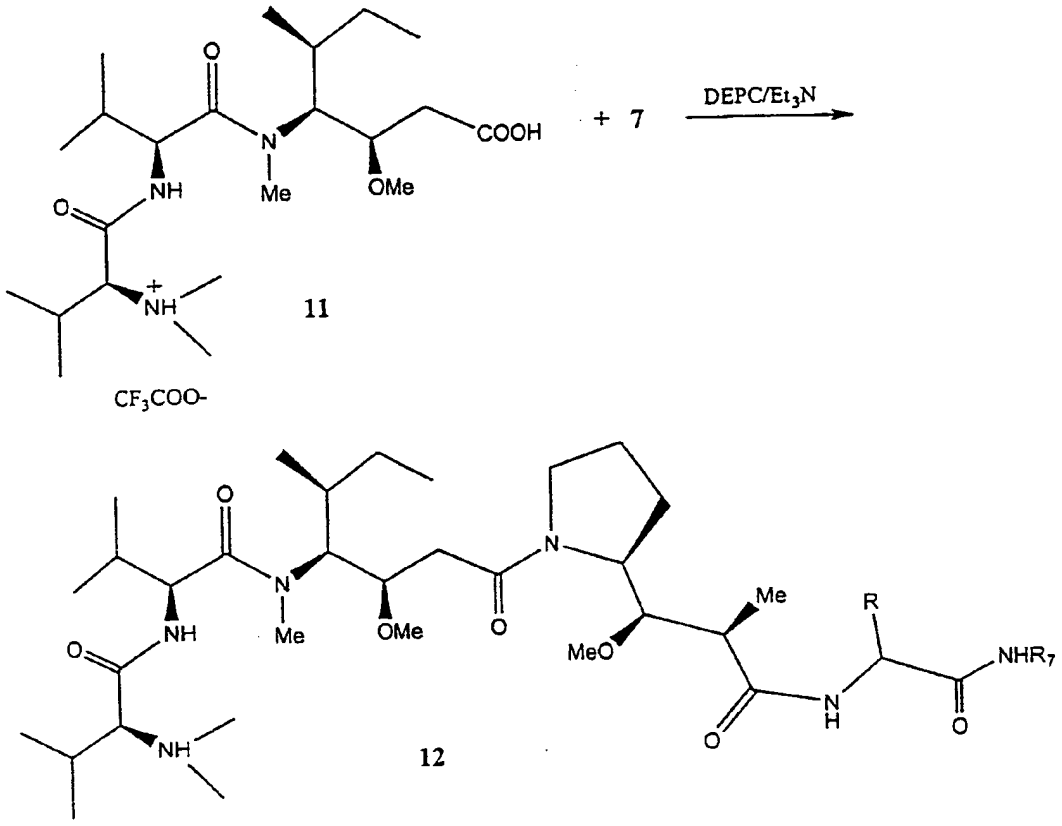
i) diethyl phosphorocyanidate (DEPC), triethylamine, dichloromethane

ii) trifluoroacetic acid, dichloromethane

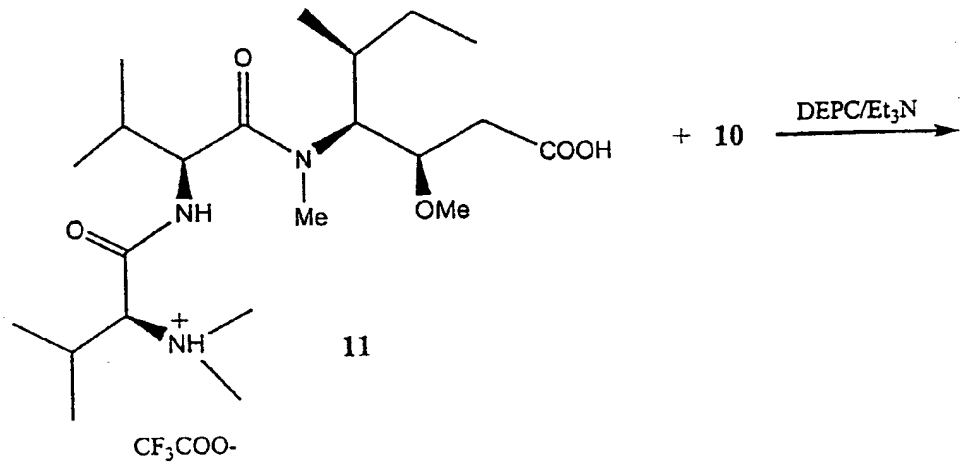
Table 5. Physical constants and spectroscopic data for the tetrapeptide amides 13a-g

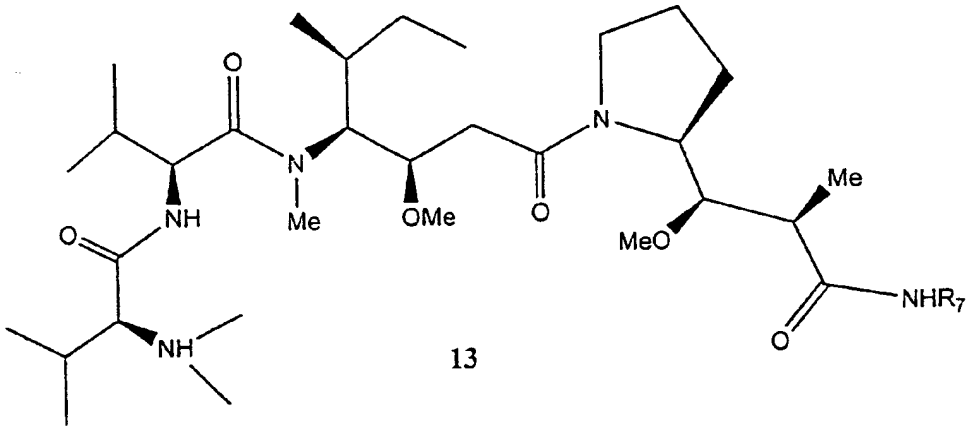
no.	R_7	yield %	mp °C	R_f	$[\alpha]_D^{25}$ °CHCl$_3$	ir, νmax, cm^{-1}	^{1}H nmr, δ	ms M$^+$	Molecular Formula
13a	o	62	85-90	0.29 (3:2 acetone-hexane)	-22 (c 0.14)	3291 1670 1647	7.79(d), 4.63-4.8(m), 4.0(m), 3.85(m), 3.39/3.38(s), 3.34/3.31/3.3(s), 2.99(s), 2.83(bs)	715	$C_{35}H_{65}N_5O_8S$
13c	p	70	90-98	0.46 (3:2 acetone-hexane)	-93 (c 0.06)	3293 1701 1624	7.15(d), 4.76(m), 4.6(m),. 4.23(m), 4.07(m), 3.87(dd), 3.71(t), 3.39/3.32/3.31(s), 2.98(s), 2.34(s), 1.26(d)	697	$C_{35}H_{63}N_5O_7S.3H_2O$
13d	q	45	115-122	0.56 (3:2 acetone-hexane)	-45 (c 0.1)	3256 1672 1626	8.33(s), 7.94(s), 4.63(s), 3.96(s), 3.43/3.41(s), 3.39(s)/3.31(s), 3.03(s)	852	$C_{40}H_{62}N_6O_7F_6.4H_2O$
13e	j	34	-	0.25 (1:1 acetone-hexane)	-37 (c 0.26)	1622	4.79, 4.87(q), 4.12(m), 3.92(dd), 3.4/3.41(s), 3.31/3.33(s)	733	$C_{41}H_{75}N_5O_6.H_2O$
13f	k	97	75-80	0.35 (1:1 acetone-hexane)	-21 (c 2.7)	1640	7.25(d), 4.68-4.77(m), 4.25(m), 4.10(m), 3.86(d), 3.40/3.32(s), 3.01(s), 1.25(d)	731	$C_{41}H_{73}N_5O_6$
13g	r	50	83-88	0.52 (2:3 acetone-hexane)	-37 (c 2.1)	1669 1632	8.54(s), 8.27(d), 7.95(dd), 6.92(m), 6.65(d), 4.77(m), 4.01(d), 3.46(s), 3.34(s), 3.02(s)	825	$C_{42}H_{70}N_7O_6F_3$

Scheme III



Scheme IV


O
N
COOH
+ 10
DEPC/Et3N
NH
Me
OMe
O
+
NH
11
CF3COO-


O
N
N
Me
NH
Me
OMe
O
MeO
NHR7
O
NH
13

inorganic salts were collected and the organic layer was concentrated and chromatographed on a silica gel column using 2:1 hexane-acetone as eluent to yield the required amide as a colorless solid (16d, 0.82 g, 55%): R_f = 0.6 (3:2 hexane-ethyl acetate); $[\alpha]_D^{25}$ = -44° (c 0.12, chloroform); mP 56-60°C; IR(neat): 3271, 3194, 2976, 2928, 1682, 1537, 1437, 1392, 1368, 1285, 1231, 1163, 1049, 1024 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 5.25(m, 1H, NH), 4.60(m, 1H, C^{α}-H), 2.83(s, 3H, ArS-Me), 2.82(t, 2H, S-CH_2), 2.15-2.30(m, 1H, 1/2CH_2), 2.09(s, 3H, ArS-Me), 1.95-2.05(m, 1H, 1/2CH_2), 1.65(s, 1H, NH), 1.44(s, 9H, *t*-Bu); MS(m/z): 378(M^+), 304, 278, 204, 174, 131, 104 and 57(100%).

Synthesis of the other amides 16a-c, e-g were all carried out in the same manner as described above.

Example 10 - Synthesis of the dipeptide amides 19a-g

Synthesis of Boc-Dap-Phe amide 19d

A solution of *t*-Boc-phenylalanine amide (100 mg, 0.25 mM) in dry dichloromethane (2 ml) trifluoroacetic acid (2 ml) was stirred at 0°C for 2 hr under argon. The solvent was removed *in vacuo* and the reside dissolved in toluene and reconcentrated twice. The oily trifluoroacetate salt 17d was dried under high vacuum.

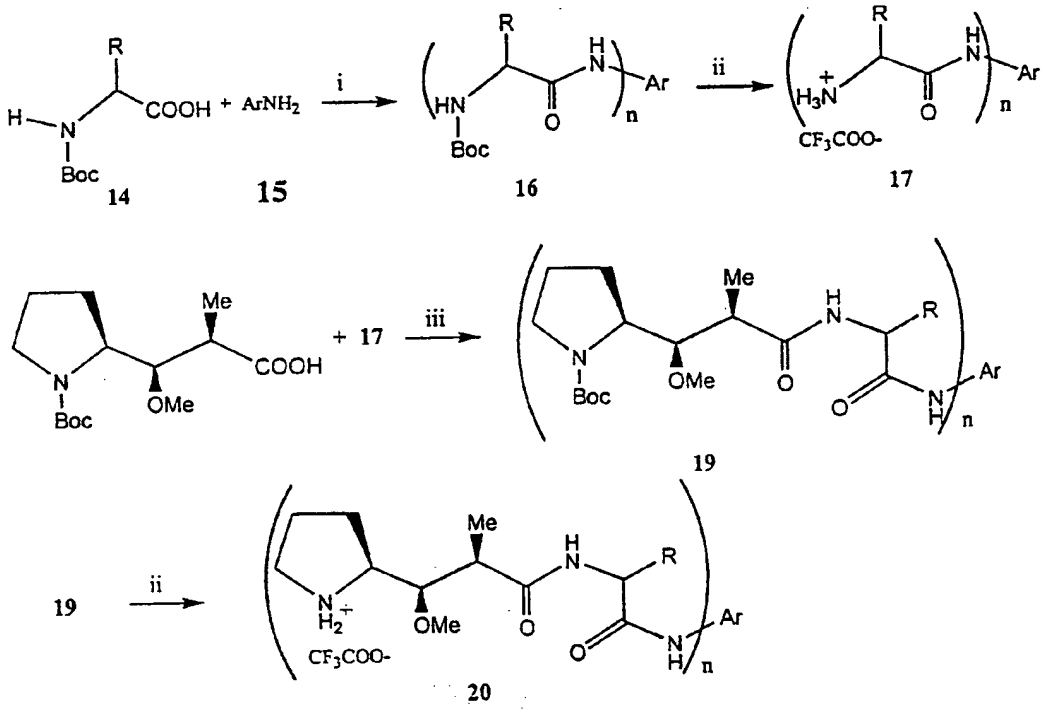
To a solution of the above trifluoroacetate salt and t-Boc-dolaproine (5, 75 mg, 0.26 mM) in dry dichloromethane (3 ml) cooled to 0°C, was added triethylamine (145 µl, 4 eq.) followed by diethyl phosphorocyanidate (DEPC, 50 µl, 1.2 eq.). The mixture was stirred for 2 hr at 0°C. The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column with 2:1 hexane-acetone as the eluent to afford the required dipeptide amide as a colorless solid (19d, 93mg, 69%) ; mP 49-52°C; R_f=0.28 (1:2 acetone-hexane); $[\alpha]_D^{25}$=-72.7° (c 0.11, chloroform); IR(thin film): 3306, 3292, 3277, 3190, 3179, 3061, 3032, 2976, 2932, 2880, 1690, 1656, 1651, 1547, 1501, 1478, 1454, 1402, 1368, 1321, 1229, 1169, 1115, 1065 and 1034 cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$): 7.21-7.32(m, 5H, Ph), 6.95(brd, 1H, NH), 4.84(m, 1H, C^{α}-H), 4.20(m, 1H, C^{α}-H), 3.37(s, 3H, O-Me), 2.60(s, 3H, S-Me), 1.45(s, 9H, But), 1.05(d, *J 7.Hz*, 3H, CH_3); MS(m/z): 531(M^+), 505, 490, 431, 394, 379, 350, 210, 170 and 114(100%).

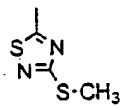
The general procedures of Examples 9 and 10 are depicted in Scheme V.

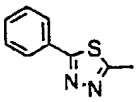
Table 6. Physical and spectroscopic data for the t-Boc-amino acid amides 16a-g.

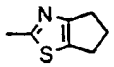
no.	n	R	Ar	yield %	mp°C	R_f	$[\alpha]_D^{25}$ $CHCl_3$	ir, νmax, cm^{-1}	^1H nmr, δ	ms M^+
16a	1	$(CH_2)_2SMe$	s	83	174-175	0.37 (3:2 hexane-ethyl-acetate)	-91 (c 0.2)	3308(br) 1717 (br)	7.45(d, NH), 4.62 (m, C^{α}-H), 2.70(t, S-CH_2), 2.04(s, 3H, S-Me)	408
16b	1	$(CH_2)_2SMe$	t	12	-	0.52 (3:2 hexane-ethyl-acetate)	-40 (c 0.12)	3217 (br) 1682 (br)	5.25 (m, NH), 4.60 (m, C^{α}-H), 2.83 (s, S-CH_2), 2.82 (t, S-CH_2), 2.09 (s, S-Me)	378
16c	1	$(CH_2)_2SMe$	u	52	146-149	0.43 (7:3 hexane-acetone)	-51 (c 0.16, MeOH)	3217 (br) 1713, 1688	5.25 (m, NH), 4.50 (m, C^{α}-H), 2.86(t), 2.74(t), 2.56(t), 2.07(s), 1.43(s)	371
16e	1	CH_2Ph	t	88	196-198	0.45 (3:2 hexane-ethyl-acetate)	-62 (c 0.38)	3297 (br) 1715 (br)	7.10 (m, NH), 4.80 (m, C^{α}-H), 3.30 (dd, 1H), 3.05 (dd, 1H), 1.19 (s, Bu^t)	424
16f	2	$(CH_2)_2SMe$	v	76	98-99	0.17 (3:1 hexane-acetone)	-45.5 (c 1.0)	3297 (br) 1667 (br)	7.40 (d), 7.06(d), 4.44(m), 3.87(s), 2.09(s), 1.40(s)	660
16g	2	$(CH_2)_2SMe$	w	52	-	0.19 (3:1 hexane-acetone)	-7.4 (c 0.38)	3308 (br) 1692 (br)	7.72(d), 7.58(dd) 4.46(m), 2.09(s), 1.41(s), 0.90(d)	571

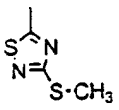
Scheme V

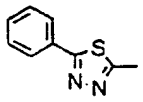


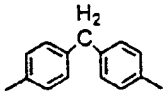
a) n=1; R = -$(CH_2)_2$SMe; Ar = 

b) n=1; R = -$(CH_2)_2$SMe; Ar = 

c) n=1; R = -$(CH_2)_2$SMe; Ar = 

d) n=1; R = -CH_2Ph; Ar = 

e) n=1; R = -CH_2Ph; Ar = 

f) n=2; R = -$(CH_2)_2$SMe; Ar = 

g) n=2; R = -$(CH_2)_2$SMe. Ar = 

i) ethyl chloroformate, triethylamine, dichloromethane

ii) trifluoroacetic acid, dichloromethane

iii) diethylphosphorocyanidate (DEPC), triethylamine, dichloromethane

Table 7. Physical and spectroscopic data for the t-Boc-Dap-amino acid amides 19a-g.

no.	n	R	Ar	yield %	mp °C	R_f	$[\alpha]_D^{25}$ Chloroform	ir, νmax, cm^{-1}	^1H nmr, δ	ms, M^+
19a	1	$(CH_2)_2SMe$	s	69	49-52	0.28 (1:2 acetone-hexane	-72.7 (c 0.11)	3306 (br) 1690, 1656, 1651	7.38(d), 4.75(m), 4.28(m), 3.45(s), 2.59(s), 2.12(s), 1.45(s)	557
19b	1	$(CH_2)_2SMe$	t	81	-	0.3 (1:2 acetone-hexane)	-48.2 (c 0.11)	3325 (br) 1692, 1597, 1582	4.83(m), 3.88(m), 3,78(s), 2.71(s), 2.07(s), 1.45(s)	577
19c	1	$(CH_2)_2SMe$	u	56	164-167	0.4 (3:7 acetone-hexane)	-69.3 (c 0.43, MeOH)	3190, 1692, 1651	7.36(bs), 6.86(bs), 4.84(m), 3.40(s), 1.98(s), 1.43(s)	540
19e	1	CH_2Ph	t	74	79-82	0.32 (1:2 acetone-hexane)	-43.8 (c 0.21)	3295 (br) 1692 (br)	7.86(d), 7.49(m), 7.27(s), 5.05(s), 3.25(s), 1.46(s)	593
19f	2	$(CH_2)_2SMe$	v	20	207-209	0.73 (8:1 dichloromethane-methanol	-120 (c 0.02)	3289 (br) 1692, 1636, 1607	7.51(d), 7.05(m), 4.65(m), 3.41(s), 2.11(s), 1.41(s)	579 (M^+ -419)
19g	2	$(CH_2)_2SMe$	w	40	65-69	0.07 (1:3 acetone-hexane)	-53.5 (c 0.17)	3306 (br) 1692, 1667	7.57-7.65 (b), 7.76(d), 4.67(m), 3.42(s), 2.01(s) 1.43(s)	909

Example 11 - Synthesis of N-Boc-dolaproine amides 22a-h N-t-Boc-Dolaproine-2-(p-aminophenyl)ethylamide 22d

To a solution of Boc-dolaproine (0.3 g, 1.05 mmole) and *p*-amino-phenethylamine (0.15 ml, 1.1 eq) in dry dichloromethane (15 ml) at 0°C under nitrogen was added triethylamine (0.44 ml, 3 eq.) followed by diethyl phosphorocyanidate (0.22 ml, 1.4 eq.). After stirring for 1 hr, the solvent was removed *in vacuo*. The residue was purified by flash chromatography on a silica gel column using 3:7 acetone-hexane to get the required amide as a clear liquid (22d, 0.56 g, 100%); R_f=0.34 (1:1 acetone-hexane); $[\alpha]_D^{25}$= -43° (c 0.34, MeOH); IR(neat): 3341, 2972, 2934, 2876, 1667, 1547, 1518, 1454, 1406, 1366, 1256, 1169, 1107 cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$): 6.97(bs), 6.61(d), 3.52(t), 3.47(t), 3.37(s), 1.56(m), 1.47(bd), 1.36(m); MS(m/z): 405(M^+), 373, 332, 287, 261, 255, 221, 187, 170, 159, 138, 119(100%).

This general procedure is depicted in Scheme VI.

Example 12 - Synthesis of tripeptides (26a-c)

Synthesis of Diethyl Val-Leu-Dil-COOBu^t 26b

N-Z-(S)-Leu-Dil-OBu^t (24b, 0.12 g, 0.237 mM) was dissolved in anhydrous methanol (5 ml) under nitrogen. Cyclohexene (5 ml) was added followed by Pd-C (5%, 0.12 g) and the solvent was immediately heated to reflux. The solution was maintained at reflux for 6 min, cooled, filtered through celite and concentrated to a clear oil which was dried under vacuum for 2h.

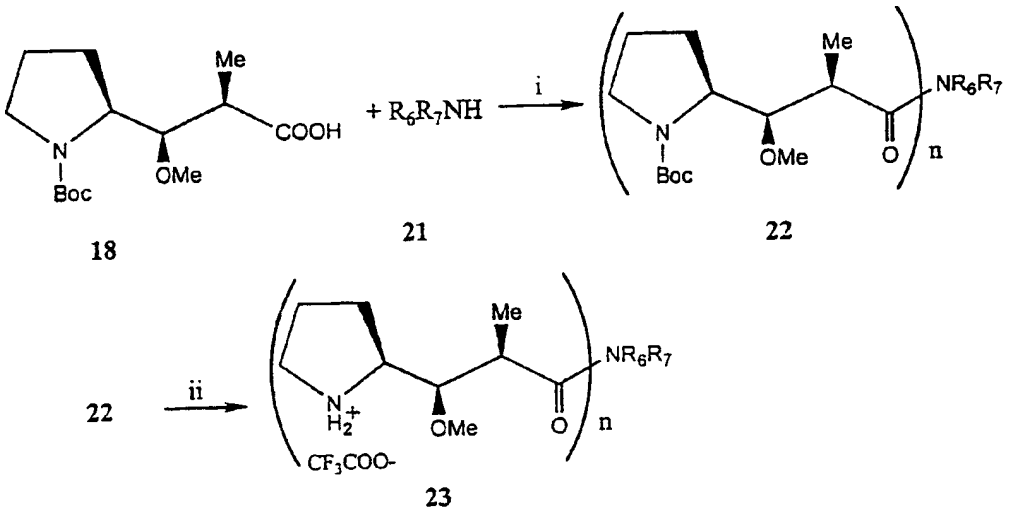
N,N-diethyl-valine (25b, 0.05 g, 0.285 mmol) was dissolved in dry dichloromethane (5 ml) under nitrogen. The solution was cooled to 0°C and triethylamine (0.04 ml, 0.284 mM) was added followed by DEPC (0.04 ml, 0.28 mM). The dipeptide was added to this mixture, the solution was allowed to warm to ambient temperature, and stirred for 1h. The mixture was concentrated under reduced pressure and chromatographed over silica gel (3:17 acetone-hexane) to give the tripeptide as a clear liquid (24b, 0.129 g, 96%); R_f=0.73(1:3 acetone-hexane); $[\alpha]_D^{25}$= -47.8° (c 0.13, MeOH); IR(neat): 3308, 2965, 1730, 1628, 1524, 1468, 1290, 1155, 1103 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 6.69(bd), 4.97(m), 3.85(m), 3.31(s), 1.43(s), 0.96(t); MS(m/z): 527(M^+), 485, 457, 270, 242, 186, 128(100%) and 100.

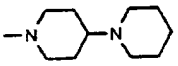
This procedure is depicted in Scheme VII.

Table 8. Physical and spectroscopic data for the t-Boc-Dap-amides 22a-h.

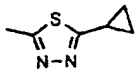
no.	n	R_6	R_7	yield %	mp °C	R_f	$[\alpha]_D^{25}$ °Chloro-form	ir, νmax, cm^{-1}	^1H nmr, δ	ms M$^+$
22a	1	H	a	82	-	0.45 (5:1 dichloro-methane-methanol)	-50.8 (c 0.13)	3497 (br) 1692 1643	3.42 (s, OMe), 1.18 (d, 6.6Hz, Me)	437
22b	1	H	b	64	-	0.33 (1:1 acetone-hexane)	-35.0 (c 0.14, Methanol)	3351 (br) 1690, 1528	10.06 (NH), 8.80(d), 8.76(d), 8.14(d), 7.49(t), 7.42(t), 3.51(s), 1.45(s)	413
22c	1	H	d	81	-	0.44 (1:1 acetone-hexane)	-50.3 (c 0.3, Methanol)	3157 (br) 1694 1549	3.49(s), 2.96(m), 2.29(m), 1.44(s), 1.33(d)	410
22e	1	H	g	17	-	0.48 (1:1 acetone-hexane)	-31.0 (c 0.21, Methanol)	3319 1688 1516	6.96(d), 6.63(d), 4.78(m), 4.19(t), 3.50(s), 1.46(s)	405 M$^+$ -FMOC
22f	1	i	COOMe	88	-	0.75 (1:1 acetone-hexane)	-17.6 (c 0.37, Methanol)	3308 1670 1543	7.95(m), 7.24(m), 6.85(d), 3,86(s), 3.35(s), 1.80(m), 1.47(s), 1.19(d)	459
22g	1	$-CH_2Ph$	c	84	104-106	0.25 (2:1 hexane-acetone)	-6.3 (c 0.16)	3308 (br) 1746 1686	8.73(d), 7.05(m), 6.83(m), 4.89(m), 3.69(s), 3.38(s), 1.47(s), 1.19(d)	455
22h	2	NR_6R_7=	f	82	-	0.29 (3:2 hexane-acetone)	-53.2 (c 0.22)	1692 1645	3.42(s, O-Me), 1.2(d, 6.8Hz, Me), 1.47(s)	624

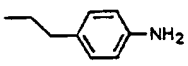
Scheme VI



a) n=1; R_6 = H; R_7 = 

b) n=1; R_6 = H; R_7 = 

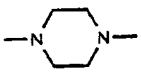
c) n=1; R_6 = H; R_7 = 

d) n=1; R_6 = H; R_7 = 

e) n=1; R_6 = H; R_7 = 

f) n=1; R_6 =  ; R_7 =COOMe

g) n=1; R_6 = CH_2Ph; R_7= 

h) n=2; R_6R_7N= 

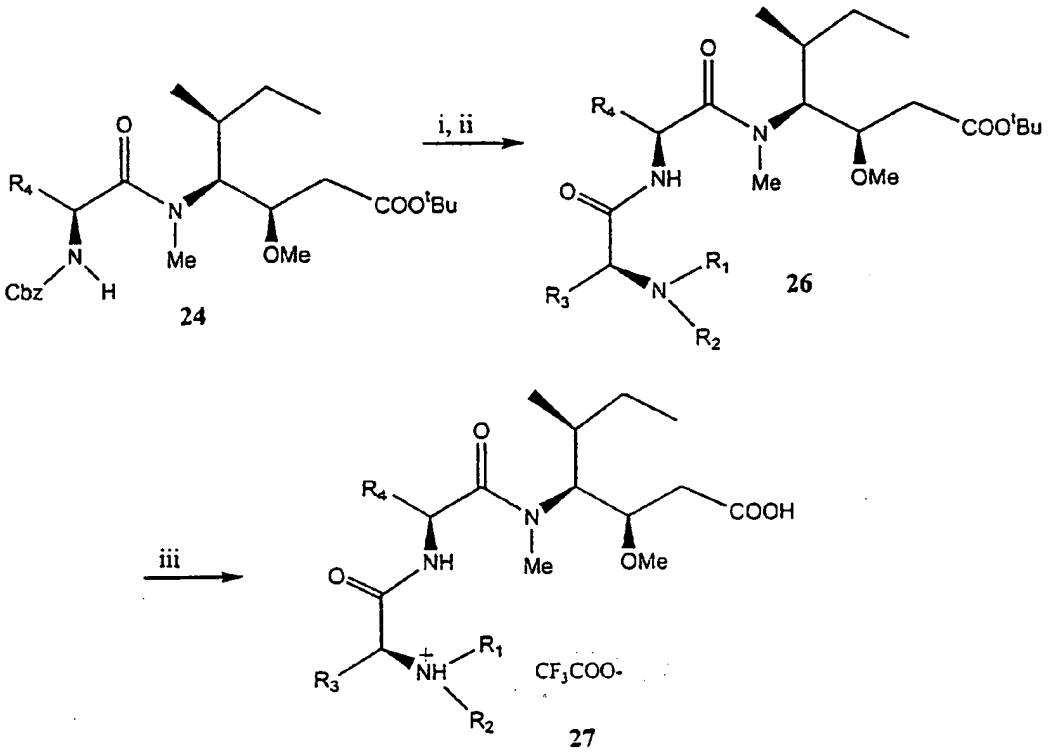
i) ethyl chloroformate, triethylamine, dichloromethane

ii) trifluoroacetic acid, dichloromethane

Table 9. Physical and spectroscopic data for tripeptide 26c

R_4	R_3	R_1, R_2	yield %	R_f	$[\alpha]_D^{25}$ °	ir, νmax, cm^{-1}	^1H nmr, δ	ms, M^+
Bu^i	Bu^t	Me	64	0.51 (1:3 acetone-hexane)	-29.3 (c 0.8, methanol)	3308 (br) 1730, 1628	6.89(bd), 4.96(m), 3.86(m), 3.32(s), 1.44(s)	513

Scheme VII



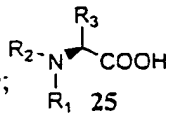
a) R_4=Pr^i; R_3=Pr^i; R_1=R_2=Me

b) R_4=Bu^i; R_3=Pr^i; R_1=R_2=Et

c) R_4=Bu^i; R_3=Bu^s; R_1=R_2=Me

i) H_2/Pd-C, cyclohexene, methanol

ii) DEPC, triethylamine, dichloromethane;


25

iii) trifluoroacetic acid, dichloromethane

Example 13 - Synthesis of pentapeptide amides 28a-g

Synthesis of Dov-Val-Dil-Dap-Phe amide 28d

To a solution of the dipeptide amide (20d, 30 mg, 0.057 mM) in dichloromethane (1 ml) cooled to 0°C under argon was added trifluoroacetic acid (1 ml). The solution was stirred at the same temperature for 2 hr. Solvent was removed *in vacuo* and the residue was dissolved in toluene and reconcentrated twice. The oily trifluoroacetate salt was dried *in vacuo*.

To a solution of the above salt and the tripeptide trifluoroacetate salt (Tfa* Dov-Val-Dil-COOH, 27a, 31 mg, 0.057 mM) in dry dichloromethane (2 ml) cooled to 0°C (under argon) was added triethylamine (32 µl, 4 eq) followed by DEPC (11.5 µl, 1.2 eq.). The solution was stirred at the same temperature for 2 hr. Solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column using 2:1 acetone-hexane as the solvent: $[\alpha]_D^{25}$= -50° (c 0.1, chloroform); mP 88-92°C; IR(thin film): 3291, 2963, 2932, 2876, 2832, 1622, 1549, 1499, 1452, 1416, 1387, 1267, 1229, 1200, 1171, 1099 and 1038 cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$): 7.20-7.30(m, Ph), 5.04-5.10(m), 4.75-4.87(m), 4.57(m), 3.38(s), 3.35(s), 3.33(s), 3.31(s), 3.14(s), 3.07(s), 2.61(s); MS(m/z): 874(M^+).

This procedure is depicted in Scheme VIII.

Example 14 - Synthesis of tetrapeptide amides 29a-l

Synthesis of Dov-Val-Dil-Dap 2-[p-aminophenyl]ethylamide 29d

A solution of the dipeptide Boc-Dap-2-p-amino-phenylethylamide (22d, 0.56 g, 1.38 mM) in dichloromethane (35ml) was cooled to 0°C (under nitrogen). Triethylamine (0.4 ml, 2.1 eq) was added followed by Fmoc-Cl (0.75 g, 2.1 eq) and the solution was stirred at room temperature for 30 min. Solvent was removed under reduced pressure and the residue chromatographed on a silica gel column using acetone-hexane (1:9 to 1:1 gradient) as the solvent to afford the required Fmoc protected peptide (0.43 g, 50%).

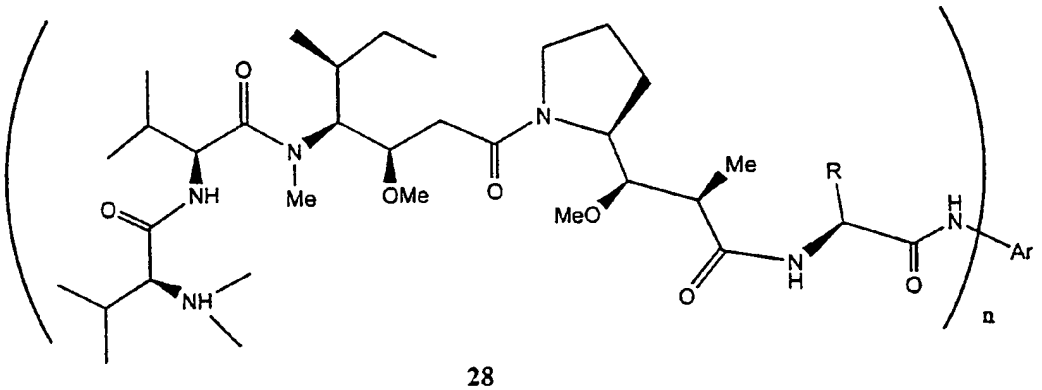
A solution of the above compound (0.38 g, 0.61 mM) in dichloromethane (0.5 ml) was cooled to 0°C under nitrogen and trifluoromethane (0.5 ml) was added. The solution was stirred at the same temperature for 1 hr. The solvent was removed and the residue dried *in vacuo*. To a solution of the trifluoroacetate salt and the tripeptide trifluoroacetate salt (27a, 0.38 g, 0.61 mM) in dry dichloromethane (5 ml),

Table 10. Physical and spectroscopic data for the dolastatin analogs 28a-g

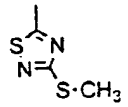
no.	n	R	Ar	yield%	mp °C	R_f	$[\alpha]_D^{25}$ °Chloroform	ir, νmax, cm^{-1}	^1H nmr, δ	ms, M^+	Molecular Formula
28a	1	$(CH_2)_2SMe$	s	48	110-116	0.5 (3:2 acet/hex)	-34.7 (c 0.32)	3275 1643 1620	4.80, 3.44, 3.32, 2.59, 2.12	858	$C_{39}H_{70}N_8O_7S_3$
28b	1	$(CH_2)_2SMe$	t	36	130-135	0.36 (3:2 acet/hex)	-51 (c 0.1)	3293 1622	7.87-7.93, 7.44, 3.44, 3.37, 3.34, 3.29, 3.09, 3.04, 2.13, 2.10	888	$C_{44}H_{72}N_8O_7S_2.2.5H_2O$
28c	1	$(CH_2)_2SMe$	u	65	79-83	0.20 (1:1 acet/hex)	-65 (c 0.18, methanol)	3271 1649 1622	4.78, 3.50, 3.36, 3.32, 3.28, 3.11, 3.04, 2.07	851	$C_{42}H_{73}N_7O_7S_2$
29e	1	CH_2Ph	t	75	123-126	0.33 (2:1 acet/hex)	-52.9 (c 0.14)	3291 1622	7.86-7.93, 7.45, 7.26 3.35, 3.32, 3.31, 3.11, 3.03	904	$C_{48}H_{72}N_8O_7S_2$
28f	2	$(CH_2)_2SMe$	v	62	107-115	0.45 (8:1 dichloro-methane-methanol)	-47.5 (c 0.08)	3385 1643 1624	7.37, 7.04, 3.39, 3.28, 2.94, 2.10, 2.23	1620	$C_{85}H_{144}N_{12}O_{14}S_2$
28g	2	$(CH_2)_2SMe$	w	17	106-110	0.28 (2:1 acet/hex)	-55.0 (c 0.06)	3291 1642 1626	3.38, 3.35, 3.33, 2.99, 2.23, 2.10	1533 $(M+H)^+$	$C_{77}H_{137}N_{13}O_{14}S_2$

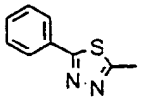
Scheme VIII

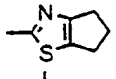
20 + n 27 ⟶

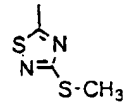


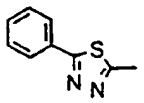
28

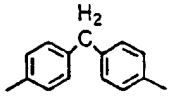
a) n=1; R = -$(CH_2)_2$SMe; Ar = 

b) n=1; R = -$(CH_2)_2$SMe; Ar = 

c) n=1; R = -$(CH_2)_2$SMe; Ar = 

d) n=1; R = -CH_2Ph; Ar = 

e) n=1; R = -CH_2Ph; Ar = 

f) n=2; R = -$(CH_2)_2$SMe; Ar = 

g) n=2; R = -$(CH_2)_2$SMe, Ar = 

i) diethylphosphorocyanidate (DEPC), triethylamine, dichloromethane

cooled to 0°C under nitrogen, was added DEPC (0.14 ml, 1.5 eq) followed by triethylamine (0.42 ml, 5.0 eq). The solution was stirred at the same temperature for 1h and allowed to come to room temperature. Removal of solvent *in vacuo* gave a residue which was subjected to flash chromatography on a silica gel column with acetone-hexane (1:1) as the eluent to provide the Fmoc protected tetrapeptide amide which was deprotected by stirring at room temperature with diethylamine (0.3ml) in dichloromethane (10 ml) for 2 hr. The product was purified by flash chromatography on a silica gel column using acetone-hexane (1:4 to 7:3 gradient) to get the free amine as a white solid (29a, 0.24 g, 54%); R_f = 0.21 (1:1 acetone-hexane); $[\alpha]_D^{25}$ = -20° (c 0.38, methanol); mP 83-86 °C; IR(thin film): 3306, 2965, 2920, 2876, 2832, 1622, 1518, 1451, 1418, 1385, 1202, 1099, 1036 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 6.97(d), 6.60(d), 6.37(m), 4.77(m), 3.35(t), 3.30(s), 3.13(s), 3.01(s), 2.68(t), 2.25(s); MS(m/z): 716(M^+), 673, 628, 525, 481, 449, 390, 227, 186, 170, 154, 119, 100 (100%).

This procedure is depicted in Scheme IX.

Example 16

Preparation of BOC-DAP-Amine 30:

A solution of Boc-DAP (0.20g, 0.70mmol) in dry methylene chloride (15 mL) under N_2 was cooled to 0°C and triethylamine (0.29 mL, 3.0 eq.) was added. DEPC (0.15ml, 1.4 eq.) was added and the reaction was stirred for 5 minutes. t-Butylamine (0.08ml, 1.1 eq.) was added and the solution was stirred at 0°C for 3 hr. The solvent was then removed under reduced pressure and the product was purified via flash chromatography (30% Acetone / Hexane) to afford 0.17g (70%) of the desired amide.

^1H NMR:300Mhz ($CDCl_3$) δ 6.31 (bs, 1H), 4.21 (m, 1H), 3.44 (s, 3H), 3.40 (m, 1H), 3.32-3.21 (m, 2H), 2.01-1.65 (m, 5H), 1.43 (m, 9H), 1.38 (s, 9H), 1.21 (bd, 3H). Mass spectrum: $C_{18}H_{34}N_2O_4$ 310 (M^+-MeOH), 269, 263, 210, 170, 154, 114, 110, 86, 84, 70 (100), 58, 50, 42. IR (neat): 3351, 2976, 2936, 2882, 1694, 1535, 1454, 1393, 1370, 1285, 1258, 1167 cm^{-1}. Rotation:-37 (C = 1.8 mg, MeOH)

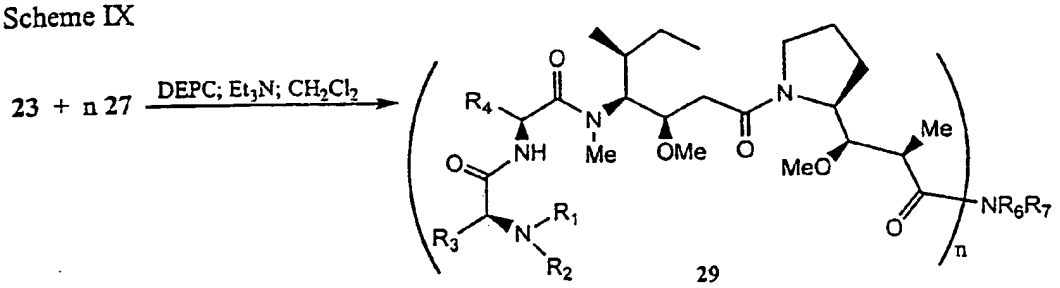
Table 11. Physical constants and spectroscopic data for the dolastatin 10 structural modifications 29a-1

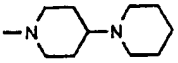
no.	n	R_6	R_7	R_4	R_3	R_1, R_2	yield %	mp °C	R_f	$[\alpha]_D^{25}$ °Chlor-oform	ir, νmax, cm^{-1}	^1H nmr, δ	ms, M^+
29a	1	H	a	Pr^i	Pr^i	Me	33	100-105	0.34 (5:1 dichloro-methane-methanol)	-132 (c 0.05)	3511 1620	3.53, 3.51, 3.49, 3.24, 2.38	748
29b	1	H	b	Pr^i	Pr^i	Me	77	73-76	0.32 (1:1 acetone-hexane)	-30.6 (c 0.17)	3295 1686 1624	10.18, 8.8 8.14, 7.48 4.76, 3.52 3.48, 3.37 3.27, 2.98 2.23	724
29c	1	H	d	Pr^i	Pr^i	Me	56	77-80	0.11 (1:1 acetone-hexane)	-38.6 (c 0.5, methanol)	3165 1620	4.76, 3.55 3.37, 3.19 3.03, 2.34 1.38	721
29e	1	H	g	Bu^s	Pr^i	Me	62	85	0.16 (1:1 acetone-hexane)	-16.3 (c 0.08, methanol)	3306 1622	6.98, 6.60 4.80, 3.36 3.30, 3.02 2.71, 2.24	730

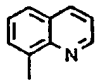
Table 11. *contd*

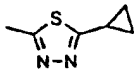
29f	1	H	g	Pr^i	Pr^i	Me	91	-	0.27 (1:1 acetone-hexane)	-20.0 (c 0.09, methanol)	3308 1676	7.58, 7.11 4.70, 3.75 3.48, 3.42 2.98, 2.80 2.23	716
29g	1	H	h	Pr^i	Pr^i	Me	38	101-105	0.19 (1:1 acetone-hexane)	-13.3 (c 0.09, methanol)	3291 1620	8.61, 7.2 7.02, 6.8 4.74, 3.81 3.31, 3.3 2.97, 2.25	770
29h	1	H	h	Bu^s	Pr^i	Me	38	105	0.2 (1:1 acetone-hexane)	-8.0 (c 0.1, methanol)	3289 1678 1626	8.42, 7.20 7.02, 6.8 4.8, 3.82 3.31, 3.3 2.29	752 M'-MeOH
29i	1	i	COO Me	Pr^i	Pr^i	Me	66	61-65	0.6 (3:1 acetone-hexane)	-15.3 (c 0.15)	3297 1748 1622	3.73, 3.68 3.37, 3.35 3.32, 3.29 3.12, 2.99 2.23	766
29j	1	$PhCH_2$	c	Bu^s	Pr^i	Et	82	65-70	0.66 (2:1 acetone-hexane)	-55.0 (c 0.06)	3293 1626	7.71-7.74, 7.17-7.26, 5.52-5.65, 4.99, 3.39 3.35, 3.32 3.31, 2.98	826
29k	1	$PhCH_2$	c	Bu^s	Bu^i	Me	82	68-75	0.51 (3:2 acetone-hexane)	-61.8 (c 0.11)	3291 1643	7.71, 3.37 3.33, 2.96	812
29l	2	NR_6R_7=	f	Pr^i	Pr^i	Me	86	112-115	0.45 (9:1 methanol $CHCl_3$)	-65.8 (c 0.12)	3380 1655 1640 1628	3.40, 3.37 3.30, 3.12 2.99	1246

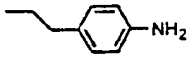
Scheme IX

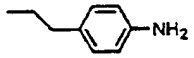


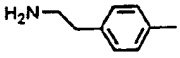
a) n=1; R_6 = H; R_7 =  ; R_4=R_3=Pr^i; R_1=R_2=Me

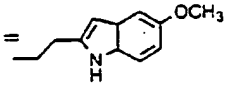
b) n=1; R_6 = H; R_7 =  ; R_4=R_3=Pr^i; R_1=R_2=Me

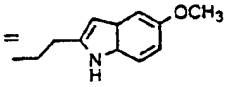
c) n=1; R_6 = H; R_7 =  ; R_4=R_3=Pr^i; R_1=R_2=Me

d) n=1; R_6 = H; R_7 =  ; R_4=R_3=Pr^i; R_1=R_2=Me

e) n=1; R_6 = H; R_7 =  ; R_4=Bu^s; R_3=Pr^i; R_1=R_2=Me

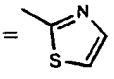
f) n=1; R_6 = H; R_7 =  ; R_4=R_3=Pr^i; R_1=R_2=Me

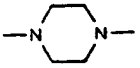
g) n=1; R_6 = H; R_7 =  ; R_4=R_3=Pr^i; R_1=R_2=Me

h) n=1; R_6 = H; R_7 =  ; R_4=Bu^s; R_3=Pr^i; R_1=R_2=Me

i) n=1; R_6 =  ; R_7 =COOMe ; R_4=R_3=Pr^i; R_1=R_2=Me

j) n=1; R_6 = -CH_2Ph; R_7 =  ; R_4=Bu^i; R_3=Pr^i; R_1=R_2=Et

k) n=1; R_6 = -CH_2Ph; R_7 =  ; R_4=Bu^i; R_3=Bu^s; R_1=R_2=Et

l) n=2; R_6R_7N=  ; R_4=Bu^i; R_3=Bu^s; R_1=R_2=Me

Preparation of DOV-VAL-DIL-DAP-t-butylamide 31:

Boc-DAP-t-butylamide 17 (0.19 g, 0.54 mmol) was dissolved in anhydrous methylene chloride (1 mL) under N_2 and cooled to 0°C. Trifluoroacetic acid (1 ml) was added and the solution was stirred at 0°C for 2 hours. The solvents were removed under a stream of N_2 after warming to room temperature and the remaining residue was desiccated under vacuum for 2 hours. Tripeptide (1.0 eq., DOV-VAL-DIL-OtBu) was deprotected concurrently using the same procedure.

The resulting salts were combined in 5 mL of anhydrous methylene chloride under N_2. The solution was cooled to 0°C and triethylamine (0.23 mL, 3.0 eq.) was added followed by diethylcyanophosphonate (0.11 mL, 1.3 eq.). The solution was stirred at 0°C for 1 hour and then allowed to warm to room temperature and stirred an additional 2 hours. The mixture was concentrated under reduced pressure and chromatographed over silica gel (9:1 CH_2Cl_2 / MeOH) to furnish the desired derivative 0.08g (23%). Mass spectrum: $C_{35}H_{67}O_6N_5$ 653 (M^+), 638, 610, 578, 525, 481, 449, 428, 327, 227, 199, 186, 154, 128, 100 (100), 85. IR (neat): 3306, 2965, 2932, 2876, 1622, 1535, 1452, 1416, 1366, 1200, 1099 cm^{-1}. Rotation:-46 (C= 1.2 mg, MeOH). mP. 120 - 125°C

Example 17

Preparation of Boc-dolaproine-isopropyl amide, 32

To a solution of Boc-Dap (145 mg, 0.51 mmol) in methylene chloride (10 mL) cooled to 0°C was added HOBt (75 mg), EDC (105 mg) and triethylamine (85 µl). After 1 hr, isopropylamine (50 µl) was added and the solution was stirred for 1 hr at 0°C, followed by 15 hr at room temp. The thin layer chromatogram of the reaction mixture (2:3 ethyl acetate-hexane) indicated the formation of the product (R_f 0.21). The reaction was diluted with methylene chloride (5 ml), washed successively with 10% citric acid (10 ml), water (10 ml), satd $NaHCO_3$ solution (10 ml), and water (10 ml) and dried over anhydrous $MgSO_4$. The thin layer chromatogram of the solution indicated a single product which was collected by concentrating the solution and drying under vacuum. Yield was 120 mg (72%); $[\alpha]_D^{25}$ -44.4° (c, 0.378, $CHCl_3$).

Preparation of Dov-Val-Dil-Dap-isopropylamide, 33

A stirred solution of Boc-Dap-isopropylamide (33 mg, 0.1 mmol) in methylene chloride (1 mL) and trifluoroacetic acid (1 ml) in an ice bath was allowed to react for 2 hr, then solvents were removed *in vacuo*. The residue was dissolved in toluene and reconcentrated. The TFA salt was dried under vacuum for 24 hr. Tripeptide (Dov-Val-Dil-OtBu 54.3 mg) was deprotected concurrently using the same procedure.

The resulting salts were combined in methylene chloride (2 mL) and cooled to 0°C. Triethylamine (50 µL) was added followed by diethylcyano phosphonate (23 µL). The solution was stirred at 0°C for 2 hr. Solvents were removed under vacuum and the residue was chromatographed on silica gel (8:1 CH_2Cl_2-MeOH) to provide a pale yellow solid, 60 mg (96% yield): $[\alpha]_D^{25}$ -47.1° (c, 0.104, $CHCl_3$), m.p. 70-73 °C, R_f0.37 (3:2 acetone-hexane).

Preparation of BOC-DAP-Amine 20

A solution of Boc-DAP (0.21g, 0.71mmol) in dry methylene chloride (15ml) under N_2 was cooled to 0c and triethylamine (0.25ml, 2.5 eq.) was added. DEPC (0.15g, 1.4 eq.) was added and the reaction was stirred for 5 minutes. Methylamine (0.43ml of a 2.0 M solution in CH_2Cl_2, 1.2 eq.) was added and the solution was stirred at 0°C for 2 hours. The solvent was removed under reduced pressure and the product was purified via flash chromatography (20% Acetone/Hexane) to afford 0.19 g (90%) of the desired amide. Mass spectrum: $C_{15}H_{28}N_2O_4$ 268 (M^+-MeOH), 227, 210, 170, 168, 157, 154, 131, 116, 114, 110, 100, 73, 70 (100), 58. IR (neat) 3308, 2974, 2936, 2880, 1694, 1651, 1549, 1456, 1402, 1366, 1254, 1167, 1105 cm^{-1}. Rotation: -26 (C=1.8mg, MeOH).

Preparation of Dov-Val-Dil-Dap-methylamide 35

Boc-DAP-methylamide (0.10g, 0.32 mmol) was dissolved in anhydrous methylene chloride (1 mL) under N_2 and cooled to 0°C. Trifluoroacetic acid (1 mL) was added and the solution was stirred at 0°C for 2 hours. The solvents were removed under a stream of N_2 after warming to room temperature and the remaining residue was desiccated under vacuum for 2 hours. Tripeptide (1.0 dq., Dov-Val-Dil-OtBu) was deprotected concurrently using the same procedure.

The resulting salts were combined in 5 mL of anhydrous methylene chloride under N_2. The solution was cooled to 0°C and triethylamine (0.14 ml, 3.0 eq.) was added followed by diethylcyanophosphonate (0.06 ml, 1.3 eq.). The solution was stirred at 0°C for 1 hour and then allowed to warm to room temperature and stirred an additional 1 hour. The mixture was concentrated under reduced pressure and chromatographed over silica gel (9:1 CH_2Cl_2 / MeOH) to furnish the desired derivative, 0.16 g (82%). Mass spectrum: $C_{32}H_{61}O_6N_5$ 611 (M^+), 596, 580, 568, 536, 525, 481, 449, 412, 386, 285, 255, 227, 199, 186, 170, 154, 128, 100 (100). IR (neat): 3304, 2963, 2936, 2876, 2832, 2789, 1622, 1532, 1452, 1416, 1200, 1099 cm^{-1}. Rotation: -27 (C=1.3mg, MeOH).

Example 18 - *In vitro* evaluation of compounds 12, 13, 28 and 29

Compounds prepared according to Examples 1-14 above were evaluated for in vitro cytotoxicity against a panel of cultured cancer cells, including the cell lines OVCAR-3 (ovarian cancer), SF-295 (central nervous system), A498 (renal cancer), NCI-H460 (non-small lung carcinoma), KM20L2 (colon cancer) and SK-MEL-5 (melanoma). For each cell line, each compound was tested at 5 concentrations, 100 μg/mL, 10 μg/mL, 1 μg/mL, 0.1 μg/mL and 0.01 μg/mL. Percent growth values were calculated for each concentration, and the two or three concentrations with growth values above, below or near 50% growth (relative to control) were used to calculate the ED_{50} value using a linear regression calculation. In cases in which 50% growth inhibition was not observed for any of the concentrations, the ED_{50} value was expressed as $ED_{50} > 100$ μg/mL. If the growth inhibition was greater than 50% for each concentration, the ED_{50} was expressed as < 0.01 μg/mL. Similar calculations were performed for total growth inhibition (TGI; 0% growth) and LC_{50} (-50% growth).

At the start of each experiment, cells from the in vitro cell culture were inoculated into tubes or microtiter plates. One set of control tubes/plates was immediately counted to determine the cell count at the beginning of the experiment. This is the "baseline count" or T_0 reading. After 48 hours, a second set of control tubes/plates is analyzed to determine the control growth value. The growth or death of cells relative to the T_0 value is used to define the percent growth. The in vitro activity data for compounds 12, 13, 28 and 29 are presented in Tables 12 and 13.

Table 12. Human Cancer and Murine P-388 Lymphocytic Leukemia (ED_{50}) Cell Line inhibitory Results for Peptides 12 & 13

Cell type	Cell line		12a	12b	12c	12d	12e	13a	13b
Ovarian	OVCAR-3	GI-50 (μg/ml)	$3.5x10^{-4}$	$3.0x10^{-4}$	$<1x10^{-4}$	$3.1x10^{-4}$	$3.5x10^{-3}$	$8.3x10^{-4}$	$3.5x10^{-4}$
CNS	SF-295		$1.1x10^{-3}$	$3.6x10^{-4}$	$1.1x10^{-2}$	$4.7x10^{-4}$	$4.3x10^{-2}$	$>1x10^{-2}$	$5.2x10^{-4}$
Renal	A498		$5.8x10^{-4}$	$3.3x10^{-4}$	$6.1x10^{-3}$	$4.8x10^{-4}$	$2.9x10^{-2}$	$3.4x10^{-3}$	$2.0x10^{-3}$
Lung-NSC	NCI-H460		$4.9x10^{-4}$	$3.3x10^{-4}$	$4.2x10^{-5}$	$2.9x10^{-4}$	$2.3x10^{-3}$	$2.9x10^{-3}$	$4.7x10^{-4}$
Colon	KM20L2		$3.8x10^{-4}$	$3.7x10^{-4}$	$1.3x10^{-4}$	$3.0x10^{-4}$	$9.1x10^{-4}$	$2.6x10^{-3}$	$3.6x10^{-4}$
Melanoma	SK-MEL-5		$2.9x10^{-4}$	$4.6x10^{-4}$	$4.0x10^{-5}$	$4.4x10^{-4}$	$4.5x10^{-4}$	$1.1x10^{-3}$	$7.0x10^{-4}$
Ovarian	OVCAR-3	TGI (μg/ml)	$1.8x10^{-3}$	$>1x10^{-2}$	$2.1x10^{-3}$	$>1x10^{-2}$	$1.0x10^{-1}$	$>1x10^{-2}$	$3.4x10^{-3}$
CNS	SF-295		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Renal	A498		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Lung-NSC	NCI-H460		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$4.0x10^{-3}$	1.1	$>1x10^{-2}$	$>1x10^{-2}$
Colon	KM20L2		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$9.0x10^{-4}$	$7.2x10^{-1}$	$>1x10^{-2}$	$>1x10^{-2}$
Melanoma	SK-MEL-5		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Ovarian	OVCAR-3	LC-50 (μg/ml)	$>1x10^{-2}$	$>1x10^{-2}$	>1	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
CNS	SF-295		$>1x10^{-2}$	$>1x10^{-2}$	>1	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Renal	A498		$>1x10^{-2}$	$>1x10^{-2}$	>1	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Lung-NSC	NCI-H460		$>1x10^{-2}$	$>1x10^{-2}$	>1	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Colon	KM20L2		$>1x10^{-2}$	$>1x10^{-2}$	>1	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Melanoma	SK-MEL-5		$>1x10^{-2}$	$>1x10^{-2}$	>1	$>1x10^{-2}$	>10	$>1x10^{-2}$	$>1x10^{-2}$
Mouse Leukemia	P-388	ED50 (μg/ml)	$4.4x10^{-3}$	$4.0x10^{-3}$	$3.0x10^{-1}$	$<1x10^{-4}$	$3.0x10^{-1}$	$7.2x10^{-3}$	$2.2x10^{-3}$

Table 12. *cont'd*

Cell type	Cell line		13c	13d	13e	13f	13g
Ovarian	OVCAR-3	GI-50 (μg/ml)	$3.1x10^{-4}$	$2.7x10^{-3}$	$1.3x10^{-3}$	$1.2x10^{-3}$	$2.3x10^{-2}$
CNS	SF-295		$1.7x10^{-3}$	$>1x10^{-2}$	$4.9x10^{-4}$	$2.6x10^{-3}$	$3.5x10^{-2}$
Renal	A498		$6.9x10^{-4}$	$>1x10^{-2}$	$3.4x10^{-3}$	$5.2x10^{-3}$	$5.6x10^{-2}$
Lung-NSC	NCI-H460		$3.7x10^{-4}$	$3.9x10^{-2}$	$2.7x10^{-3}$	$3.6x10^{-3}$	$3.1x10^{-2}$
Colon	KM20L2		$3.3x10^{-4}$	$3.6x10^{-3}$	$3.1x10^{-4}$	$4.5x10^{-4}$	$2.3x10^{-2}$
Melanoma	SK-MEL-5		$2.2x10^{-4}$	$5.6x10^{-3}$	$2.0x10^{-3}$	$2.3x10^{-3}$	$3.5x10^{-2}$
Ovarian	OVCAR-3	TGI (μg/ml)	$1.8x10^{-3}$	$>1x10^{-2}$	$6.5x10^{-3}$	$2.5x10^{-2}$	$1.3x10^{-1}$
CNS	SF-295		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Renal	A498		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Lung-NSC	NCI-H460		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Colon	KM20L2		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$1.1x10^{-1}$	$1.6x10^{-1}$
Melanoma	SK-MEL-5		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Ovarian	OVCAR-3	LC-50 (μg/ml)	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
CNS	SF-295		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Renal	A498		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Lung-NSC	NCI-H460		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Colon	KM20L2		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Melanoma	SK-MEL-5		$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	>1	>1
Mouse Leukemia	P-388	ED50 (μg/ml)	$2.5x10^{-3}$	$1.9x10^{-1}$	$4.8x10^{-3}$	$3.8x10^{-2}$	$3.5x10^{-1}$

Table 13. Human Cancer-Cell line and P-388 Mouse Leukemia (ED_{50}) data for peptides 28a-g & 29a-l

	Cell type	Cell Line	28a	28b	28c	28d	28e	28f	28g
GI-50 (μg/ml)	Ovarian	OVCAR-3	$3.1x10^{-5}$	$4.6x10^{-5}$	$4.9x10^{-5}$	$3.0x10^{-7}$	$3.6x10^{-5}$	$1.8x10^{-5}$	$9.1x10^{-4}$
	CNS	SF-295	$1.9x10^{-4}$	$3.8x10^{-4}$	$4.7x10^{-4}$	$6.1x10^{-7}$	$5.9x10^{-5}$	$>1.0x10^{-4}$	$>1x10^{-2}$
	Renal	A498	$3.8x10^{-4}$	$3.9x10^{-4}$	$2.2x10^{-4}$	$3.4x10^{-6}$	$5.3x10^{-4}$	$>1.0x10^{-4}$	$3.0x10^{-3}$
	Lung-NSC	NCI-H460	$1.1x10^{-4}$	$5.5x10^{-4}$	$4.0x10^{-4}$	$4.1x10^{-7}$	$1.9x10^{-5}$	$3.3x10^{-5}$	$2.3x10^{-3}$
	Colon	KM20L2	$1.5x10^{-4}$	$2.2x10^{-4}$	$4.5x10^{-5}$	$2.0x10^{-7}$	$3.2x10^{-6}$	$2.2x10^{-5}$	$2.4x10^{-3}$
	Melanoma	SK-MEL-5	$4.7x10^{-5}$	$7.0x10^{-4}$	$3.7x10^{-5}$	$5.6x10^{-7}$	$2.0x10^{-5}$	$4.7x10^{-6}$	$4.4x10^{-4}$
TGI (μg/ml)	Ovarian	OVCAR-3	$1.0x10^{-3}$	$7.0x10^{-3}$	$>1x10^{-2}$	$1.1x10^{-5}$	$7.9x\ 10^{-4}$	$9.4x10^{-5}$	$>1x10^{-2}$
	CNS	SF-295	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
	Renal	A498	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$2.3x10^{-4}$	$>1x10^{-4}$	$>1x10^{-2}$
	Lung-NSC	NCI-H460	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$2.1x10^{-4}$	$>1x10^{-4}$	$>1x10^{-2}$
	Colon	KM20L2	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$4.1x10^{-6}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
	Melanoma	SK-MEL-5	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$			
LC-50 (μg/ml)	Ovarian	OVCAR-3	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
	CNS	SF-295	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
	Renal	A498	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
	Lung-NSC	NCI-H460	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
	Colon	KM20L2	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
	Melanoma	SK-MEL-5	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$	$>1x10^{-4}$	$>1x10^{-2}$
ED50 (μg/ml)	Mouse Leukemia	P-388	$<1.0x10^{-3}$	$1.96X10^{-3}$	$2.03x10^{-3}$	$2.55x10^{-6}$	$8.22x10^{-5}$	$2.12x10^{-2}$	$2.05x10^{-2}$

Table 13. *cont'd*

	Cell type	Cell Line	29a	29b	29c	29d	29e	29f	29g
GI-50 (μg/ml)	Ovarian	OVCAR-3	3.2×10^{-3}	2.5×10^{-3}	3.6×10^{-2}	5.0×10^{-5}	$<1.0\times10^{-4}$	3.6×10^{-2}	$<1.0\times10^{-4}$
	CNS	SF-295	3.6×10^{-2}	1.5×10^{-3}	4.8×10^{-2}	5.3×10^{-4}	2.1×10^{-4}	2.1×10^{-1}	$<1.0\times10^{-4}$
	Renal	A498	8.1×10^{-3}	8.8×10^{-3}	1.0×10^{-1}	$>1\times10^{-2}$	9.4×10^{-4}	1.1×10^{-1}	$<1.0\times10^{-4}$
	Lung-NSC	NCI-H460	2.4×10^{-3}	2.9×10^{-3}	3.1×10^{-2}	1.3×10^{-4}	7.5×10^{-5}	1.1×10^{-1}	$<1.0\times10^{-4}$
	Colon	KM20L2	3.0×10^{-3}	1.4×10^{-3}	1.4×10^{-2}	4.9×10^{-5}	$<1.0\times10^{-4}$	4.0×10^{-2}	$<1.0\times10^{-4}$
	Melanoma	SK-MEL-5	2.8×10^{-3}	3.6×10^{-4}	3.4×10^{-2}	2.3×10^{-4}	$<1.0\times10^{-4}$	5.5×10^{-2}	$<1.0\times10^{-4}$
TGI (μg/ml)	Ovarian	OVCAR-3	1.1×10^{-2}	2.3×10^{-2}	2.9×10^{-1}	7.9×10^{-4}	1.4×10^{-3}	1.5×10^{-1}	$<1.0\times10^{-4}$
	CNS	SF-295	>1	>1	>10	$>1\times10^{-2}$	>1	>1	2.8×10^{-1}
	Renal	A498	>1	>1	>10	$>1\times10^{-2}$	3.7×10^{-1}	>1	3.4x10
	Lung-NSC	NCI-H460	9.2x10-3	1.9×10^{-1}	1.5	8.7×10^{-4}	81.1×10^{-1}	>1	>1
	Colon	KM20L2	>1	1.4×10^{-1}	1.1	$>1\times10^{-2}$	1.1×10^{-1}	>1	1.7×10^{-4}
	Melanoma	SK-MEL-5	>1	>1	>10	$>1\times10^{-2}$	>1	>1	>1
LC-50 (μg/ml)	Ovarian	OVCAR-3	>1	>1	>10	$>1\times10^{-2}$	>1	>1	>1
	CNS	SF-295	>1	>1	>10	$>1\times10^{-2}$	>1	>1	>1
	Renal	A498	>1	>1	>10	$>1\times10^{-2}$	>1	>1	>1
	Lung-NSC	NCI-H460	>1	>1	>10	$>1\times10^{-2}$	>1	>1	>1
	Colon	KM20L2	>1	>1	>10	$>1\times10^{-2}$	>1	>1	>1
	Melanoma	SK-MEL-5	>1	>1	>10	$>1\times10^{-2}$	>1	>1	>1
ED50 (μg/ml)	Mouse Leukemia	P-388	5.11×10^{-2}	3.53×10^{-3}	2.72×10^{-1}	3.38×10^{-4}	3.56×10^{-3}	4.01×10^{-2}	1.84×10^{-3}

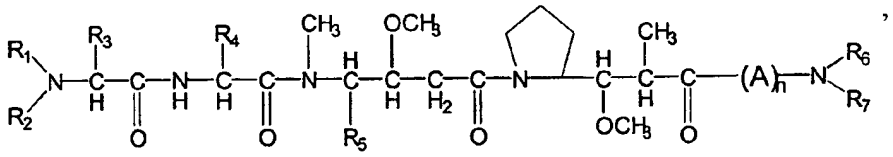
Table 13. *cont'd*

	Cell type	Cell Line	29h	29i	29j	29k	29l
GI-50 (μg/ml)	Ovarian	OVCAR-3	$<1.0\times10^{-4}$	3.4×10^{-4}	4.7×10^{-5}	3.1×10^{-4}	1.6×10^{-2}
	CNS	SF-295	2.5×10^{-4}	2.6×10^{-4}	2.8×10^{-4}	4.0×10^{-4}	3.8×10^{-1}
	Renal	A498	7.1×10^{-4}	$>1\times10^{-3}$	2.7×10^{-4}	3.2×10^{-4}	8.4×10^{-2}
	Lung-NSC	NCI-H460	1.1×10^{-4}	3.0×10^{-4}	1.0×10^{-4}	2.9×10^{-4}	3.0×10^{-2}
	Colon	KM20L2	$<1.0\times10^{-5}$	3.9×10^{-5}	4.7×10^{-5}	3.4×10^{-5}	3.4×10^{-2}
	Melanoma	SK-MEL-5	$<1.0\times10^{-4}$	1.5×10^{-4}	5.9×10^{-5}	2.3×10^{-4}	5.8×10^{-3}
TGI (μg/ml)	Ovarian	OVCAR-3	3.2×10^{-4}	$>1\times10^{-3}$	7.9×10^{-4}	$>1\times10^{-2}$	1×10^{-1}
	CNS	SF-295	2.8×10^{-1}	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
	Renal	A498	3.1×10^{-1}	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
	Lung-NSC	NCI-H460	>1	8.8×10^{-4}	1.4×10^{-3}	8.4×10^{-4}	>1
	Colon	KM20L2	1.9×10^{-2}	$>1\times10^{-3}$	$>1\times10^{-2}$	1.0×10^{-3}	>1
	Melanoma	SK-MEL-5	>1	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
LC-50 (μg/ml)	Ovarian	OVCAR-3	>1	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
	CNS	SF-295	>1	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
	Renal	A498	>1	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
	Lung-NSC	NCI-H460	>1	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
	Colon	KM20L2	>1	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
	Melanoma	SK-MEL-5	>1	$>1\times10^{-3}$	$>1\times10^{-2}$	$>1\times10^{-2}$	>1
ED50 (μg/ml)	Mouse Leukemia	P-388	3.60×10^{-3}	2.73×10^{-1}	2.11×10^{-4}	$<1\times10^{-4}$	1.66×10^{-1}

CLAIMS

What is claimed is:

1. The compound of the formula



or a salt thereof with a pharmaceutically acceptable acid, wherein

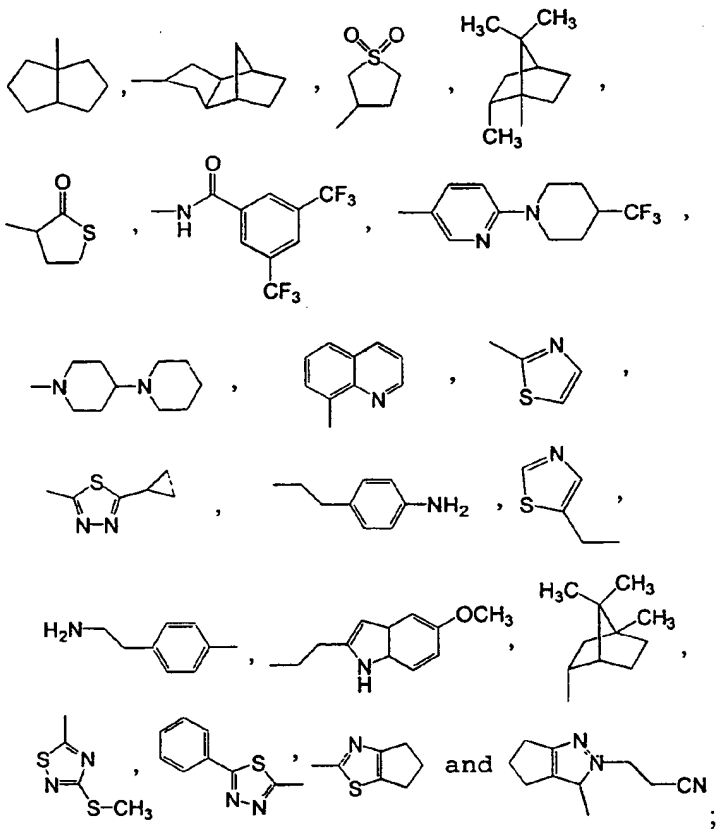
R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group;

A is a methionyl, phenylalanyl or phenylglycyl residue;

n is 0 or 1;

R_6 is a hydrogen atom; and

R_7 is selected from the group consisting of t-butyl, isopropyl, methyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-(3-pyridyl)ethyl, 4-pyridyl,



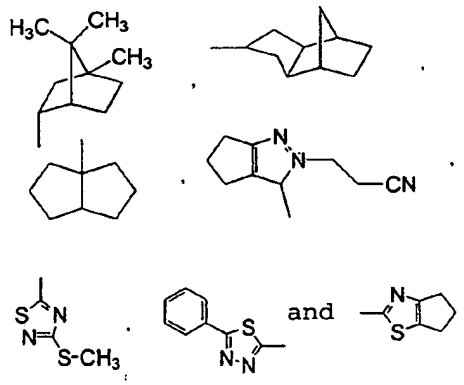
or

R_6 is benzyl or -C(O)OR_8, wherein R_8 is a C_1-C_6-alkyl group; and

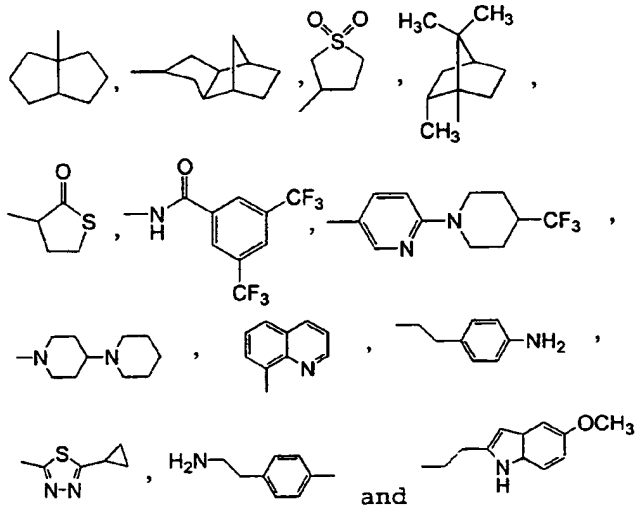
R_7 is a 2-thiazolyl group.

2. The compound of Claim 1 wherein R_1 and R_2 are each a methyl group, R_3 is an isopropyl or sec-butyl group, R_4 is an isopropyl, sec-butyl or isobutyl group, and R_5 is a sec-butyl group.

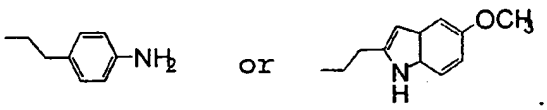
3. The compound of Claim 2 wherein R_1 and R_2 are each methyl; R_3 and R_4 are each isopropyl; R_5 is sec-butyl; n is 1; A is a methionyl residue; R_6 is a hydrogen atom; and R_7 is selected from the group consisting of



4. The compound of Claim 2 wherein R_1 and R_2 are each methyl, R_3 and R_4 are each isopropyl, R_5 is sec-butyl, n is 0, R_6 is a hydrogen atom and R_7 is selected from the group consisting of t-butyl, isopropyl, methyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-(3-pyridyl)ethyl, 4-pyridyl,

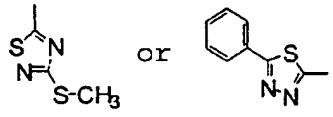


5. The compound of Claim 2 wherein R_1 and R_2 are each methyl; R_3 is isopropyl; R_4 and R_5 are each sec-butyl; n is 0; R_6 is a hydrogen atom; and R_7 is

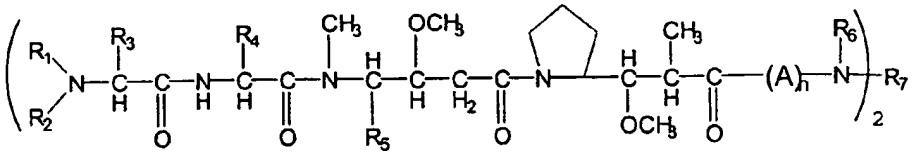


6. The compound of Claim 2 wherein R_1 and R_2 are each methyl; R_3 is isopropyl; R_4 is isopropyl or sec-butyl; R_5 is sec-butyl; n is 0; R_6 is a benzyl group or
$-C(O)OCH_3$; and R_7 is a 2-thiazolyl group.

7. The compound of Claim 2 wherein R_1 and R_2 are each methyl; R_3 is isopropyl; R_4 is isopropyl; R_5 is sec-butyl; n is 1; A is a phenylalanyl residue; R_6 is a hydrogen atom; and R_7 is



8. The compound of the formula



or a salt thereof with a pharmaceutically acceptable acid, wherein
R_1-R_5 are each, independently, a hydrogen atom or a
normal or branched C_1-C_6-alkyl group;

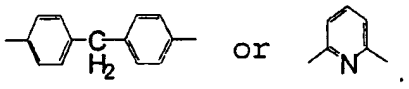
A is a methionyl, phenylalanyl or phenylglycyl residue;

n is 0 or 1;

R_6 is a hydrogen atom; and

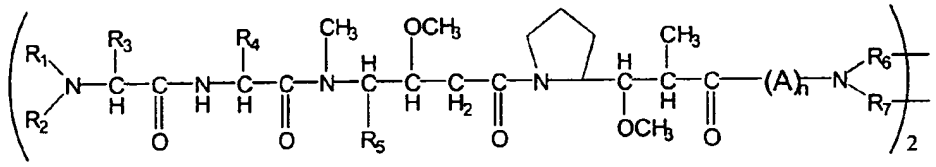
R_7 is an aromatic group.

9. The compound of Claim 8 wherein R_7 is



10. The compound of Claim 9 wherein R_1 and R_2 are each a methyl group; R_3 and R_4 are each an isopropyl group; R_5 is a sec-butyl group; n is 1; and A is a methionyl residue.

11. A compound of the formula

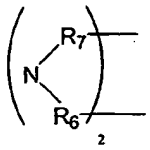


or a salt thereof with a pharmaceutically acceptable acid, wherein

R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group;

A is a methionyl, phenylalanyl or phenylglycyl residue;

n is 0 or 1; and



is a five- or six-membered ring.

12. The compound of Claim 11 wherein R_6 and R_7 are each a methylene group.

13. The compound of Claim 12 wherein R_1 and R_2 are each a methyl group; R_3 and R_4 are each an isopropyl group; R_5 is a sec-butyl group; and n is 0.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/018032 A3

(51) International Patent Classification[7]: C07K 5/02, 7/02

(21) International Application Number: PCT/US00/24658

(22) International Filing Date:
8 September 2000 (08.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/394,962	10 September 1999 (10.09.1999)	US
09/539,935	31 March 2000 (31.03.2000)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	09/394,962 (CON)
Filed on	10 September 1999 (10.09.1999)
US	09/539,935 (CON)
Filed on	31 March 2000 (31.03.2000)

(71) Applicants *(for all designated States except US)*: **BASF AKTIENGESELLSCHAFT** [DE/DE]; Rheinland-Pfalz, D-67056 Ludwigshafen (DE). **ARIZONA BOARD OF REGENTS** [US/US]; Arizona State University, Tempe, AZ 85287-3511 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PETIT, George, R.** [US/US]; 6232 Bret Hills Drive, Paradise Valley, AZ 85253 (US). **SRIRANGAM, Jayaram, K.** [IN/US]; 9395 Pipilo Street, San Diego, CA 92129 (US). **WILLIAMS, Michael, D.** [US/US]; 1544 South Vine, Gilbert, AZ 85233 (US). **DURKIN, Kieran, P., M.** [IE/US]; 110 Kerwin Way, Folsom, CA 95630 (US). **BARLOZZARI, Teresa** [IT/US]; 24 South Woodside Avenue, Wellesley, MA 02181 (US). **KLING, Andreas** [DE/DE]; Riegeler Weg 14, D-68239 Mannheim (DE). **JANSSEN, Bernd** [DE/US]; 114 Applebriar Lane, Marlborough, MA 01752 (US). **HAUPT, Andreas** [DE/DE]; Schaelzigweg 52, D-68723 Schwetzingen (DE).

(74) Agent: **DECONTI, Giulio, A.**; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

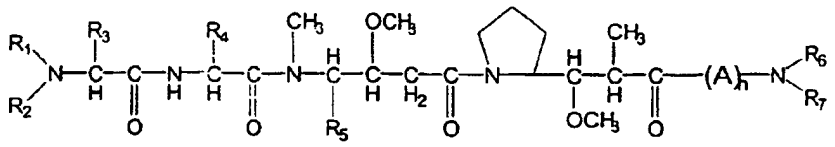
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*.

(88) Date of publication of the international search report:
11 July 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DOLASTATIN PEPTIDES



(I)

(57) Abstract: The present invention provides compounds of formula (I) where R_1-R_5 are each, independently, a hydrogen atom or a normal or branched C_1-C_6-alkyl group; A is a methionyl, phenylalanyl or phenylglycyl residue; n is 0 or 1; R_6 is a hydrogen atom; and R_7 is a carbocyclic group, an aromatic group, a C_1-C_4-alkyl group, a pyridylalkyl group or a heterocyclic group. In another embodiment, R_6 is benzyl or -C(O)OR_8, where R_8 is a C_1-C_6-alkyl group, and R_7 is a heteroaromatic group, such as a 2-thiazolyl group.

WO 01/018032 A3

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 00/24658

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K5/02 C07K7/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 939 527 A (BELIK DANIEL ET AL) 17 August 1999 (1999-08-17)	1,2,4-6
Y	column 2 -column 9	1-7
X	WO 99 35164 A (PETTIT GEORGE R ;PETTIT ROBIN K (US); UNIV ARIZONA (US)) 15 July 1999 (1999-07-15)	1-3,7
Y	claim 1	1-7
Y	PETTIT, GEORGE R. ET AL.: "ANTINEOPLASTIC AGENTS 365. DOLASTATIN 10 SAR PROBES" ANTI-CANCER DRUG DES (1998) 13(4) 243-277, XP001041934 the whole document	1-7
Y	US 5 663 149 A (SRIRANGAM JAYARAM K ET AL) 2 September 1997 (1997-09-02) claims	1-7

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 2002	11/01/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cervigni, S

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 611 775 A (UNIV ARIZONA) 24 August 1994 (1994-08-24) page 11	1-7
A	EP 0 598 129 A (TEIKOKU HORMONE MFG CO LTD) 25 May 1994 (1994-05-25)	
A	MIYAZAKI ET AL: "Synthesis and antitumor activity of novel dolastatin 10 analogs" CHEMICAL AND PHARMACEUTICAL BULLETIN, PHARMACEUTICAL SOCIETY OF JAPAN. TOKYO, JP, vol. 43, no. 10, 1995, pages 1706-1718, XP002080987 ISSN: 0009-2363	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/24658

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 5939527	A	17-08-1999	AU 4296597 A	20-02-1998
			WO 9804278 A2	05-02-1998
			EP 0920325 A2	09-06-1999
			ZA 9706723 A	12-02-1999
			ZA 9706724 A	29-01-1999
WO 9935164	A	15-07-1999	EP 1045858 A1	25-10-2000
			WO 9935164 A1	15-07-1999
US 5663149	A	02-09-1997	AU 4378196 A	03-07-1996
			CA 2203689 A1	20-06-1996
			EP 0797447 A1	01-10-1997
			JP 11503717 T	30-03-1999
			WO 9618408 A1	20-06-1996
EP 0611775	A	24-08-1994	US 5410024 A	25-04-1995
			CA 2113739 A1	22-07-1994
			EP 0611775 A2	24-08-1994
			JP 6293795 A	21-10-1994
EP 0598129	A	25-05-1994	AT 190983 T	15-04-2000
			AU 662551 B2	07-09-1995
			AU 2415292 A	02-03-1993
			DE 69230824 D1	27-04-2000
			DE 69230824 T2	27-07-2000
			DK 598129 T3	03-07-2000
			EP 0598129 A1	25-05-1994
			GR 3033397 T3	29-09-2000
			KR 185440 B1	01-04-1999
			KR 202474 B1	15-06-1999
			US 6004934 A	21-12-1999
			AU 673487 B2	07-11-1996
			AU 2001095 A	20-07-1995
			CA 2115355 A1	18-02-1993
			EP 0934950 A1	11-08-1999
			ES 2144421 T3	16-06-2000
			WO 9303054 A1	18-02-1993
			JP 2618597 B2	11-06-1997
			SG 48155 A1	17-04-1998
			US 5654399 A	05-08-1997